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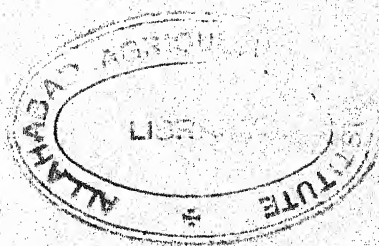
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(Vol. VI, Part I)

March, 1936

The Editorial Committee of the Imperial Council of Agricultural Research, India, takes no responsibility for the opinions expressed in this Journal.

AN EXPERIMENTAL STUDY OF RINDERPEST VIRUS IN GOATS IN A SERIES OF 150 DIRECT PASSAGES

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(With Plate I and 82 Charts.)

In connection with the problem of combating rinderpest in India, several methods of protecting cattle against the disease have been evolved from time to time, but the question of cost stood in the way of the serum-simultaneous method being adopted throughout the country, though this method seemed to offer an efficient protection. In the search for a cheaper method of affording protection against the disease what is known as " the goat-virus-alone " method has come into prominence during the last three or four years. Stirling [1932, 1933] has recorded his views on the use of goat virus-alone on a fairly large scale in the Central Provinces and Kerr and Menon [1934] have also recorded the work done with goat-virus-alone in Bengal. It appears that the initial supply of material for these goat-virus-alone inoculations was from the Imperial Institute of Veterinary Research, Muktesar. Edwards [1927] says that " prolonged and intensive research has led to means being devised whereby the virus can be easily propagated in goats by successive inoculation with small quantities of blood," but this virus was then intended only " to overcome the distressing complication often observed after the serum-simultaneous inoculation caused by the unwitting introduction of virulent piroplasms with rinderpest." Since Koch [1896-97] made his observations on the behaviour of the rinderpest virus in goats, there have been attempts in various parts of the world to find out the extent, if any, of its practical application in immunising cattle against rinderpest. Bliss [1922] in China and Topacio [1926] appear to have been the first to put it to practical use. It is generally accepted that rinderpest virus may be attenuated by successive passages through goats, yet in none of the available literature is any mention made of experimental work designed to show how and under what conditions rinderpest virus becomes attenuated through goats to such a degree as to make it safe for use on bovines as an immunising agent without the anti-serum.

A

This paper is an attempt to supply the omission and to place on record the results of an experimental study of rinderpest virus with a view to determine whether successive passages of the virus through goats would so alter the virus as to make it safe for use as a vaccine for protecting cattle against the disease without anti-serum. In other words, the aim of the experiment is to find out (1) whether there is a progressive attenuation of the virus as it passes from goat to goat ; (2) at what stage of this attenuation is the virus safe for use as a vaccine for protecting cattle against rinderpest ; (3) whether the attenuation through goats is so progressive as to render the virus innocuous after a certain stage is reached, or whether the virus after a certain stage in its attenuation becomes " fixed " in its virulence for goats and (4) whether this " fixed " virus is capable of being maintained indefinitely by passage in goats. It is obvious that these points have an important bearing on the practical application of the method, especially when it is intended to replace the serum-simultaneous inoculation which is universally acknowledged to be the best in the field for conferring lasting immunity against rinderpest.

The experiment was started in August 1932 and was so designed as to use two goats for each passage and a bovine as control. During these 34 months, 150 unbroken passages have been completed. All the goats used in this experiment were of the type commonly met with in this Presidency (Plate I, Figs. 1 and 2) and were obtained locally, and the controls were mostly buffalo-calves of local breed of the kind used at the Madras Serum Institute for to produce rinderpest bull virus for serum-simultaneous inoculations. These animals are of more than average susceptibility. A summary of the observations recorded is set out in Table I appended. As it would take up a great deal of space if the temperature charts of all the experimental animals were included, only 31 temperature charts (I—LXII) representing every fifth passage from the first to the 150th in this series are included.

DISCUSSION

It will be observed from the details recorded in the tabular statement referred to that (1) continuity of passage from goat to goat has been possible ; (2) the reactions in the goats have been constant throughout ; (3) there has been no difference in the mortality rate in goats between the earlier and later passages ; (4) there is very little variation in the clinical symptoms in the experimental goats at any stage during these 150 passages and (5) no marked variations have been observed in the *post mortem* lesions in the goats. Some of these findings are in accord with the observations of other workers, while others are at variance as will be discussed later. It will further be seen that evidence of attenuation of the virus for bovines, in its passage through goats, is furnished by the nature
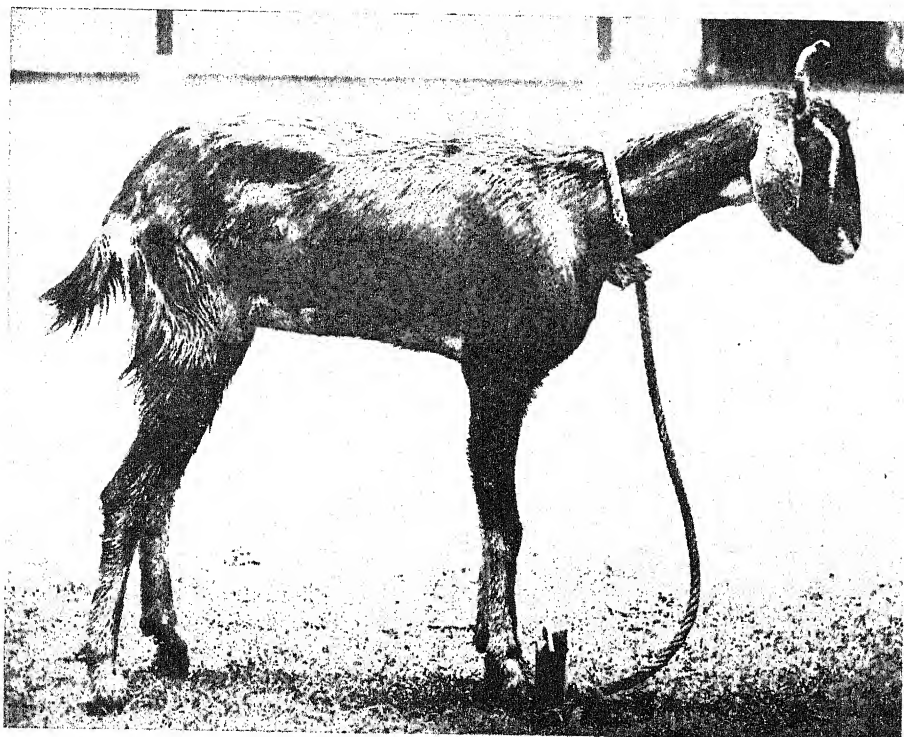
FIG. 1. Type of goat (healthy).

FIG. 2. Goats (7 days after inoculation.)

In order to compare the reaction in local goats and to study the possible variations in the course of the disease in these goats with a different strain of virus, a parallel series of experiment was started on 14th September 1933 with goat virus (spleen tissue) obtained from the Imperial Institute of Veterinary Research, Muktesar. It is not known for how long this strain of virus had been maintained through goats at Muktesar before it was received, but this virus has been put through 95 direct passages in goats at this Institute. It will be seen from the summary of reactions (Table II) and the temperature charts, (LXIII—LXXXII) that there is hardly any difference in the nature of the reactions, course of the disease and *post mortem* lesions between the two series of experimental goats.

Percentage of reaction in goats (Muktesar strain) :—

	Per cent
Temperature	67
Vesicles	3
Diarrhœa	44
Mortality	95

SUMMARY AND CONCLUSIONS

(1) Judging from the mortality rate in the controls, there is evidence of attenuation of the virus for bovines after the 80th passage through goats.

(2) There is constancy of reaction in the experimental goats in Madras with no evidence of attenuation for these animals.

(3) All the experimental goats have readily taken the infection and there is no indication so far that the virus cannot be maintained indefinitely through goats.

(4) Ideal attenuation for bovines cannot be said to have been reached until the severity of reactions and mortality rate are reduced to the level generally considered necessary for serum-simultaneous inoculation of animals.

The authors wish to acknowledge with thanks, the help rendered by Mr. G. Theophilus, Veterinary Assistant Surgeon, Serum Institute, Madras, in keeping careful records of observations throughout the course of this experiment.

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TABLE I

(Madras Strain)

Passage Number	Kind of animal	No.	Reaction	Result	Remarks
1	Goat	5	Temperature	Recovered.	
	Do.	6	Not pronounced	Do.	
	Control white calf	136	Temperature and Diarrhœa.	Do.	
2	Goat	9	Temperature and Vesicles.	Recovered.	
	Do.	10	Not pronounced	Do.	Aborted on the 12th day.
	Control white calf	153	Temperature, Vesicles and Diarrhœa.	Died	Very severe lesions. Very bad lesions in the Ileo-cæcal valve and cæcum Typical of Rinderpest.
3	Goat	13	Not pronounced	Died	Typical lesions throughout the elementary system.
	Do.	14	Temperature. Vesicles and Diarrhœa.	Died	P. M.—Typical of Rinderpest.
	Control white calf	165	Temperature	Recovered	Piro-Bigem appeared on the 8th day.
4	Goat	17	Temperature, Vesicles and Diarrhœa.	Died	11th day aborted a foetus—P. M. typical of Rinderpest
	Do.	18	Do.	Do.	P. M. Typical of Rinderpest.
	Control white calf	175	Temperature	Recovered	5th day morning Temperature 105·4°F.

TABLE I—*contd.*

Passage Number	Kind of animal	No.	Reaction	Result	Remarks
5	Goat	19	Not pronounced	Died	P. M. Typical of Rinderpest.
	Do.	20	Temperature and Diarrhoea.	Recovered	Aborted a foetus on the 11th day.
	Control white calf	187	Temperature, Vesicles and Diarrhoea.	Died	P. M.—Typical of Rinderpest.
6	Goat	23	Vesicles	Died	P. M. lesions : Typical of Rinderpest.
	Control white calf	195	Temperature, Vesicles and Diarrhoea.	Do.	Do.
7	Goat	27	Temperature, Vesicles and Diarrhoea.	Died	P. M. Lesions : Typical of Rinderpest.
	Control white calf	210	Do.	Recovered	5th day morning Temperature 105·6° F.
8	Goat	31	Not pronounced	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	32	Do.	Do.	Do.
	Control white calf	219	No reaction. (Re-tested with bull Virus—no reaction).		Non-reactor.
9	Goat	33	Temperature, Vesicles and Diarrhoea.	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	34	Not pronounced	Do.	Do.
	Control white calf	236	Temperature, Vesicles and Diarrhoea.	Do.	Do.

10	Goat . . .	35	Temperature and Diarrhoea.	Died	P. M. Lesions : Typical of Rinderpest.
	Do. . .	36	Do.	Do. . .	Do.
	Control white calf .	227	Temperature	Do. . .	Do.
11	Goat . . .	37	Temperature, Vesicles, and Diarrhoea.	Died .	P. M. Lesions : Typical of Rinderpest.
	Do. . .	38	Do.	Do. . .	Do.
	Control white calf .	253	Do.	Do. . .	Do.
12	Goat . . .	39	Temperature, Vesicles and Diarrhoea.	Died	P. M. Lesions : Typical of Rinderpest.
	Do. . .	40	Do.	Do. . .	Do.
	Control white calf .	262	Do. .	Do. . .	Micro-filoria appeared in blood on the 2nd evening. Lesions typical of Rinderpest.
13	Goat . . .	43	Temperature and Diarrhoea.	Died .	P. M. Lesion : Typical of Rinderpest aecum—Very bad.
	Do. . .	44	Temperature and Vesicles.	Do. . .	Aborted a foetus on the 4th day. P. M. typical of Rinderpest.
	Control white calf .	269	Temperature .	Do. . .	P. M. lesions : Typical of Rinderpest.
14	Goat . . .	45	Vesicles .	Died .	Good lesions in the gall-bladder. Aborted a foetus—P. M. lesions typical of Rinderpest. Good mouth lesions.
	Do. . .	46	Temperature .	Do. . .	P. M. Lesions : Typical of Rinderpest.
	Control white calf .	279	Temperature, Vesicles and Diarrhoea.	Recovered.	
15	Goat . . .	48	Vesicles and Diarrhoea	Died .	P. M. Lesions : Typical of Rinderpest.
	Control white calf .	288	Temperature, Vesicles and Diarrhoea.	Recovered.	

B

TABLE I—*contd.*

Passage Number	Kind of animal	No.	Reaction	Result	Remarks
16	Goat	50	Not pronounced	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	51	Do.	Do.	Do.
	Control buffalo calf	297	Temperature, Vesicles and Diarrhœa.	Do.	P. M. Lesions typical of Rinderpest. Good lesions in the gall-bladder.
17	Goat	52	Diarrhœa	Died	P. M. lesions : Typical of Rinderpest.
	Do.	53	Temperature and Diarrhœa.	Do.	Do.
	Control white calf	306	Temperature, Vesicles and Diarrhœa.	Recovered	4th day evening Temperature 107° F.
18	Goat	54	Diarrhœa	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	55	Temperature, Vesicles and Diarrhœa.	Do.	Do.
	Control white calf	313	Temperature	Recovered.	
19	Goat	56	Temperature and Diarrhœa.	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	57	Do.	Do.	Do.
	Control buffalo calf	318	Temperature, Vesicles and Diarrhœa.	Recovered.	

			Symptoms	Result	P. M. Lesions
20	Goat	58	Temperature and Diarrhoea.	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	59	Temperature and Vesicles.	Do.	Do.
	Control white calf	329	Temperature, Vesicles and Diarrhoea.	Do.	Do.
21	Goat	61	Temperature	Died	5th day morning Temperature : 105·4. P. M. Lesions : Typical of Rinderpest.
	Do.	62	Vesicles and Diarrhoea	Do.	Good month lesions—P. M. lesions : Typical of Rinderpest complicated with Pneumonia.
	Control buffalo calf	347	Temperature and Diarrhoea.	Do.	P. M. Lesions : Typical of Rinderpest.
22	Goat	63	Temperature and Diarrhoea.	Died	P. M. Lesions : Typical of Rinderpest complicated with Pneumonia.
	Do.	64	Do.	Do.	P. M. Lesions : Typical of Rinderpest.
	Control white calf	359	Vesicles and Diarrhoea	Do.	Piro-Bi·em appeared in blood on the 2nd day evening. P. M. Lesions : Typical of Rinderpest with Pneumonia.
23	Goat	65	Temperature	Died	P. M. Typical of Rinderpest.
	Do.	66	Temperature and Vesicles.	Do.	P. M. Typical of Rinderpest with Pneumonia.
	Control buffalo calf	370	Temperature, Vesicles and Diarrhoea.	Do.	P. M. Lesions : Typical of Rinderpest.
24	Goat	68	Not pronounced	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	69	Vesicles and Diarrhoea	Do.	Do.
	Control white calf	374	Temperature and Diarrhoea.	Recovered.	

TABLE I—*contd.*

Passage Number	Kind of animal	No.	Reaction	Result	Remarks
25	Goat	70	Temperature and Vesicles.	Died	P. M. Lesions : Typical of Rinderpest. 7th day morning Temperature : 105.6° F.
	Do.	71	Do.	Do.	P. M. Lesions : Typical of Rinderpest. with good lesions in the Abomasum.
	Control buffalo calf	381	Temperature, Vesicles and Diarrhœa.	Do.	P. M. Lesions : Typical of Rinderpest.
26	Goat	72	Not pronounced	Died	P. M. Lesions. Typical of Rinderpest.
	Do.	74	Temperature	Do.	Do.
	Control buffalo calf	390	Temperature, Vesicles and Diarrhœa.	Recovered.	
27	Goat	75	Diarrhœa	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	76	Not pronounced	Recovered.	
	Control buffalo calf	401	Temperature, Vesicles and Diarrhœa.	Do.	
28	Goat	77	Not pronounced	Recovered.	
	Do.	78	Temperature	Died	P. M. Lesions : Typical of Rinderpest.
	Control buffalo calf	407	Temperature, Vesicles and Diarrhœa.	Do.	Do.

29	79	Goat	Not pronounced	Died	P. M. Lesions : Typical of Rinderpest.
	80	Do.	Do.	Do.	Do.
	418	Control buffalo calf	Temperature, Vesicles and Diarrhoea.	Do.	P. M. Lesions : Typical of Rinderpest. Very good lesions in the Pharynx Small-Intest. Peyers' Patches, Ileo-cæcal valve, cæcum and colon.
30	82	Goat	Temperature and Diarrhoea.	Died	Ova of strongyles and amœba present. P. M. Lesions : Typical of Rinderpest.
	83	Do.	Not pronounced	Do.	P. M. Lesions : Typical of Rinderpest.
	425	Control buffalo calf	Temperature, Vesicles and Diarrhoea.	Do.	Do.
31	1	Goat	Temperature	Died	P. M. Lesions : Typical of Rinderpest.
	2	Do.	Not pronounced	Do.	Do.
	433	Control buffalo calf	Temperature, Vesicles and Diarrhoea.	Recovered.	
32	3	Goat	Temperature Vesicles.	Died	P. M. Lesions : Typical of Rinderpest.
	4	Do.	Temperature	Do.	Do.
	1	Control buffalo calf	Temperature, Vesicles and Diarrhoea.	Do.	P. M. Lesions : Typical of Rinderpest. 4th day evening Temperature : 106.6° F.
33	5	Goat	Not pronounced	Died	P. M. lesions : Typical of Rinderpest.
	6	Do.	Do.	Died	P. M. Lesions : Typical of Rinderpest.
	11	Control white calf	Temperature and Vesicles.	Recovered.	

TABLE I—*contd.*

Passage Number	Kind of animal	No.	Reaction	Result	Remarks
34	Goat	7	Not pronouced	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	8	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	14	Temperature	Do.	Do.
35	Goat	9	Temperature and Diarrhœa.	Died	P. M. Lesions : Typical of Rinderpest. Good lesions in the Abomasum.
	Do.	10	Diarrhœa.	Recovered.	
	Control buff. calf	22	Temperature, Vesicles and Diarrhœa.	Do.	
36	Goat	12	Diarrhœa.	Recovered	P. M. : Typical of Rinderpest.
	Do.	13	Temperature and Diarrhœa.	Died	
	Control buff. calf	32	Temperature, Vesicles and Diarrhœa.	Do.	Do.
37	Goat	14	Temperature, Vesicles and Diarrhœa.	Recovered	
	Do.	15	Temperature and Vesicles.	Do.	
	Control white calf	39	Temperature, Vesicles and Diarrhœa.	Do.	
38	Goat	16	Temperature, Vesicles and Diarrhœa.	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	17	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	49	Non-reactor		Non-reactor.—Retested with bull virus also.

39	Goat	18	Temperature	Died	P. M. Lesions : Rinderpest with Pneumonia.
	Do.	19	Temperature, Vesicles and Diarrhœa.	Do.	P. M. Lesions : Typical of Rinderpest.
	Control buff. calf	58	Temperature	Recovered	Microfiloria appeared on the 7th day.
40	Goat	20	Temperature, Vesicles and Diarrhœa.	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	21	Temperature, Vesicles and Diarrhœa.	Do.	Do.
	Control buff. calf	68	Temperature, Vesicles and Diarrhœa.	Recovered.	
41	Goat	22	Temperature and Diarrhœa.	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	23	Not pronounced	Do.	Do.
	Control buff. calf	76	Temperature, Vesicles and Diarrhœa.	Do.	Do.
42	Goat	24	Temperature, Vesicles and Diarrhœa.	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	25	Diarrhœa	Do.	P. M. Lesions : Rinderpest with Pneumonia.
	Control buff. calf	83	Vesicles and Diarrhœa	Recovered	
43	Goat	26	Not pronounced	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	27	Temperature	Do.	Do.
	Control buff. calf	89	Temperature and Vesicles.	Recovered.	
44	Goat	29	Temperature, Vesicles and Diarrhœa	Died	P. M. Lesions : Rinderpest with Pneumonia.
	Do.	30	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	94	Temperature, Vesicles and Diarrhœa.	Do.	P. M. Lesions : Rinderpest.

TABLE I—*contd.*

Passage Number	Kind of animal	No.	Reaction	Result	Remarks
45	Goat	31	Diarrhoea .	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	32	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	100	Temperature, Vesicles and Diarrhœa.	Do.	Do.
46	Goat	33	Temperature and Diarrhœa.	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	34	Do.	Do.	Do.
	Control buff. calf	106	Temperature. Vesicles and Diarrhœa.	Recovered.	
47	Goat	35	Temperature Vesicles.	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	36	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	111	Temperature, Vesicles and Diarrhœa.	Do.	Do. Very good lesions in small Intes. Peyers' Patches Ileo-Cæcal valves cæcum and colon.
48	Goat	38	Not pronounced .	Died	P. M. Lesions : Typical of Rinderpest.
	Control buff. calf	118	Temperature, Vesicles and Diarrhœa.	Do.	Do.

No.	Animal	No.	Symptoms	Result	Remarks
49	Goat .	39	Temperature . .	Recovered .	Aborted a foetus on the 8th day.
	Control buff. calf .	121	Temperature and Diarrhœa.	Do.	
50	Goat .	42	Diarrhœa .	Died .	P. M. Lesions : Typical of Rinderpest.
	Control buff. calf .	127	Temperature, Vesicles and Diarrhœa.	Recovered.	
51	Goat .	43	Temperature and Diarrhœa.	Died .	P. M. : Typical of Rinderpest.
	Do. .	44	Diarrhœa .	Do.	Do.
	Control buff. calf .	134	Temperature, Vesicles and Diarrhœa.	Recovered.	
52	Goat .	45	Not pronounced .	Died .	P. M. : Rinderpest.
	Do. .	46	Temperature and Diarrhœa.	Do.	P. M. : Rinderpest with Pneumonia.
	Control buff. calf .	140	Temperature, Vesicles and Diarrhœa.	Do.	P. M. : Typical of Rinderpest.
53	Goat .	47	Not pronounced .	Died .	P. M. : Rinderpest with Pneumonia.
	Do. .	48	Diarrhœa .	Do.	P. M. : Rinderpest.
	Control buff. calf .	148	Temperature, Vesicles and Diarrhœa.	Do.	P. M. : Rinderpest with Pneumonia.
54	Goat .	49	Temperature and Diarrhœa.	Died .	P. M. Lesions : Typical of Rinderpest.
	Do, .	50	Do. .	Do.	Do.
	Control buff. calf .	153	Temperature, Vesicles and Diarrhœa.	Recovered.	

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TABLE I—contd.

Passage Number	Kind of animal	No.	Reaction	Result	Remarks
55	Goat	51	Temperature and Diarrhoea.	Died	P. M. Lesions : Typical of Rinderpest
	Do.	52	Do.	Do.	P. M. : Rinderpest.
	Control buff. calf	157	Do.	Recovered.	
56	Goat	55	Temperature and Vesicles.	Died	P. M. Lesions : Typical of Rinderpest complicated with pneumonia.
	Do.	56	Do.	Do.	P. M. Lesions : Typical of Rinderpest.
	Control buff. calf	163	Temperature, Diarrhoea and Vesicles.	Do.	Do.
57	Goat	59	Temperature, Vesicles and Diarrhoea.	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	60	Temperature and Diarrhoea.	Do.	Do.
	Control buff. calf	167	Temperature.	Recovered.	
58	Goat	63	Temperature and Vesicles.	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	64	Not pronounced	Do.	Do.
	Control buff. calf	176	Temperature, Vesicles and Diarrhoea.	Recovered.	
59	Goat	67	Not pronounced	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	68	Temperature and Diarrhoea.	Do.	Do.
	Control buff. calf	180	Temperature, Vesicles and Diarrhoea.	Recovered.	

No.	Animal	No.	Symptoms	Result	P. M. Lesions
60	Goat	71	Temperature and Diarrhœa.	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	72	Do.	Do.	Do.
	Control buff. calf	184	Temperature, Vesicles and Diarrhœa.	Recovered	5th day morning temperature 106°F
61	Goat	75	Temperature and Diarrhœa.	Died	P. M. : Rinderpest with Pneumonia.
	Do.	76	Not pronounced	Do.	P. M. : Typical of Rinderpest.
	Control buff. calf	191	Temperature, Vesicles and Diarrhœa.	Do.	Do.
62	Goat	79	Diarrhœa .	Died	P. M. : Typical of Rinderpest.
	Do.	80	Temperature	Do.	Do.
	Control buff. calf	196	Temperature and Diarrhœa.	Do.	Do.
63	Goat	83	Diarrhœa	Died	P. M. : Rinderpest.
	Do.	84	Not pronounced	Do.	Do.
	Control buff. calf	200	Temperature and Diarrhœa.	Do.	Do.
64	Goat	88	Temperature	Died	P. M. : Rinderpest.
	Do.	87	Diarrhœa	Do.	Do.
	Control buff. calf	209	Not pronounced	Recovered.	
65	Goat	91	Temperature and Diarrhœa.	Died	Aborted on the 5th day. P. M. : Typical of Rinderpest.
	Do.	92	Not pronounced	Do.	Do.
	Control buff. calf	216	Temperature and Diarrhœa.	Do.	Do.

TABLE I—*contd.*

Passage Number	Kind of animal	No.	Reaction	Result	Remarks
66	Goat	95	Temperature	Died	P. M. : Rinderpest with Pneumonia.
	Do.	96	Temperature and Diarrhœa.	Do.	P. M. : Typical of Rinderpest.
	Control buff. calf	222	Temperature and Diarrhœa.	Do.	Do.
67	Goat	99	Temperature and Diarrhœa.	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	100	Not pronounced	Do.	Do.
	Control buff. calf	230	Non-reactor	·	Non-reactor. Retested also with bull—virus.
68	Goat	105	Temperature and Diarrhœa.	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	106	Diarrhœa	Do.	Do.
	Control buff. calf	234	Non-reactor.	·	Non-reactor—Retested also with bull—virus.
69	Goat	109	Temperature	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	110	Not pronounced	Do.	Do.
	Control buff. calf	241	Temperature and Diarrhœa.	Do.	Do.

		No.		Result	P. M. Lesions
70	Goat	111	Temperature and Diarrhœa.	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	112	Do.	Do.	Do.
	Control buff. calf	247	Temperature, Vesicles and Diarrhœa.	Do.	Do.
71	Goat	117	Not pronounced	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	118	Do.	Do.	Do.
	Control buff. calf	253	Temperature, Vesicles and Diarrhœa.	Do.	Do.
72	Goat	121	Temperature	Died	P. M. Lesions : Typical of Rinderpest (Aborted a foetus on the 9th day).
	Do.	122	Do.	Do.	P. M. Lesions : Typical of Rinderpest
	Control buff. calf	259	Do.	Do.	Do.
73	Goat	125	Temperature and Diarrhœa.	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	126	Diarrhœa	Do.	Do.
	Control buff. calf	266	Non-reactor		Retested with bull—virus also.
74	Goat	129	Temperature	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	130	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	274	Diarrhœa	Do.	Do.
75	Goat	131	Temperature and Diarrhœa.	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	132	Temperature	Do.	Do.
	Control buff. calf	281	Temperature and Diarrhœa.	Do.	Do.

TABLE I—contd.

Passage Number	Kind of animal	No.	Reaction	Result	Remarks
76	Goat	137	Not pronounced	Died	P. M. Lesions : Typical of Rinderpest (complicated with pneumonia).
	Do.	138	Do.	Do.	Do.
	Control buff. calf	288	Temperature and Diarrhœa.	Recovered	
77	Goat	142	Not pronounced	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	143	Temperature	Do.	Do.
	Control buff. calf	294	Temperature and Diarrhœa.	Recovered	
8	Goat	148	Not pronounced	Recovered	
	Do.	149	Temperature	Died	P. M. Lesions : Typical of Rinderpest.
	Control buff. calf	304	Vesicles and Diarrhœa	Do.	Do.
79	Goat	150	Temperature, Vesicles and Diarrhœa.	Died	P.M. Lesions : Typical of Rinderpest (complicated with pneumonia).
	Do.	151	Diarrhœa	Do.	P. M. Lesions : Typical of Rinderpest.
	Control buff. calf	313	Vesicles and Diarrhœa	Recovered	
80	Goat	156	Temperature and Diarrhœa.	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	157	Temperature and Vesicles.	Do.	Do.
	Control buff. calf	321	Temperature and Diarrhœa.	Recovered.	

No.	Animal	Number	Symptoms	Result	Remarks
81	Goat	160	Not pronounced	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	161	Temperature	Do.	Do.
	Control buff. calf	325	Temperature, Vesicles and Diarrhœa.	Do.	Do.
82	Goat	164	Temperature	Died	P. M. Lesions : Typical of Rinderpest (complicated with pneumonia).
	Do.	165	Not pronounced	Do.	P. M. Lesions : Typical of Rinderpest.
	Control buff. calf	333	Temperature and Diarrhœa.	Do.	Do.
83	Goat	168	Temperature	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	169	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	338	Temperature	Do.	Do.
84	Goat	172	Temperature and Diarrhœa.	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	173	Do.	Do.	Do.
	Control buff. calf	345	Do.	Do.	Do.
85	Goat	176	Temperature and Diarrhœa.	Died	P. M. Lesions : Typical of Rinderpest.
	Do.	177	Temperature	Do.	Do.
	Control buff. calf	353	Temperature and Diarrhœa.	Recovered	
86	Goat	3	Temperature and Diarrhœa.	Died	Lesions : Typical of Rinderpest.
	Do.	4	Temperature	Do.	Lesions : Typical of Rindepest (Aborted a foetus on the 5th day).
	Control buff. calf	5	Do.	Recovered.	

TABLE I—contd.

Passage Number	Kind of animal	No.	Reaction	Result	Remarks
87	Goat	7	Temperature	Died	Lesions : Typical of Rinderpest.
	Do.	8	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	12	Do.	Do.	Do.
88	Goat	11	Not pronounced	Died	Lesions : Typical of Rinderpest.
	Do.	12	Temperature	Do.	Do.
	Control white calf	22	Temperature and Diarrhœa.	Recovered.	
89	Goat	15	Not pronounced	Died	Lesions : Typical of Rinderpest.
	Do.	16	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	32	Temperature	Recovered.	
90	Goat	19	Temperature and Diarrhœa.	Died	Lesions : Typical of Rinderpest.
	Do.	20	Temperature	Do.	Do.
	Control buff. calf	36	Temperature and Diarrhœa.	Recovered.	
91	Goat	21	Temperature	Died	Lesions : Typical of Rinderpest.
	Do.	23	Diarrhœa	Do.	Do.
	Control buff. calf	44	Temperature, Vesicles and Diarrhœa.	Recovered.	

92	Goat .	27	Not pronounced .	Died .	Lesions : Typical of Rinderpest.
	Do. .	28	Do. .	Do. .	Do.
	Control buff. calf .	52	Vesicles and Diarrhœa.	Do. .	Do.
93	Goat .	31	Not pronounced .	Died .	Lesions : Typical of Rinderpest.
	Do. .	32	Temperature .	Recovered.	
	Control buff. calf .	59	Not pronounced .	Do.	
94	Goat .	35	Diarrhœa .	Died .	P. M. : Lesions : Typical of Rinderpest.
	Do. .	36	Temperature and Diarrhœa.	Do. .	Do.
	Control buff. calf .	66	Non-reactor .		
95	Goat .	39	Temperature and Diarrhœa.	Died .	Lesions : Typical of Rinderpest.
	Do. .	40	Temperature .	Do. .	Do.
	Control buff. calf .	72	Temperature and Diarrhœa.	Recovered.	
96	Goat .	44	Temperature .	Died .	Lesions : Typical of Rinderpest.
	Do. .	45	Not pronounced .	Do. .	Do
	Control buff. calf	82	Temperature and Diarrhœa.	Recovered.	
97	Goat .	49	Temperature and Diarrhœa.	Died .	Lesions : Typical of Rinderpest.
	Do. .	50	Temperature .	Do. .	Do.
	Control buff. calf .	89	Temperature .	Recovered.	

TABLE I—*contd.*

Passage Number	Kind of animal	No.	Reaction	Result	Remarks
98	Goat	53	Temperature	Died	Lesions : Typical of Rinderpest (Complicated with pneumonia).
	Do.	54	Temperature and Diarrhœa.	Do.	Lesions : Typical of Rinderpest.
	Control buff. calf	97	Temperature, Vesicles and Diarrhœa.	Do.	Do.
99	Goat	57	Temperature	Died	Lesions : Typical of Rinderpest.
	Do.	58	Do.	Do.	Do.
	Control buff. calf	101	Temperature and Diarrhœa.	Recovered.	
100	Goat	61	Temperature	Died	Lesions : Typical of Rinderpest.
	Do.	62	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	106	Do.	Recovered.	
101	Goat	65	Temperature	Died	Lesions : Typical of Rinderpest.
	Do.	66	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	110	Temperature	Recovered.	
102	Goat	69	Temperature and Diarrhœa.	Died	Lesions : Typical of Rinderpest.
	Do.	70	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	116	Temperature and Diarrhœa.	Recovered.	

103	Goat	73	Not pronounced	Died	P. M. Typical of Rinderpest.
	Do.	74	Temperature	Do.	Do.
	Control buff. calf	128	Temperature, and Diarrhœa.	Do.	Do.
104	Goat	77	Temperature and Diarrhœa.	Died	P. M. Typical of Rinderpest.
	Do.	78	Do.	Do.	Do.
	Control buff. calf	134	Temperature	Recovered.	
105	Goat	82	Not pronounced	Died	P. M. Typical of Rinderpest.
	Do.	83	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	139	Temperature	Recovered.	
106	Goat	86	Temperature	Died	P. M. Typical of Rinderpest.
	Do.	87	Not pronounced	Do.	Do.
	Control buff. calf	146	Do.	Recovered.	
107	Goat	91	Temperature	Died	P. M. Typical of Rinderpest.
	Do.	92	Temperature and Diarrhœa.	Do.	P. M. Typical of Rinderpest (Complicated with Pneumonia).
	Control buff. calf	153	Non-reactor	Recovered.	
108	Goat	96	Diarrhœa	Died	P. M. Typical of Rinderpest.
	Do.	97	Temperature	Do.	Do.
	Control buff. calf	161	Do.	Recovered.	

TABLE I—*contd.*

Passage Number	Kind of animal	No.	Reaction	Result	Remarks
109	Goat	100	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	101	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	167	Temperature	Recovered.	
110	Goat	107	Temperature and Diarrhœa.	Died	P. M. lesions : Typical of Rinderpest.
	Do.	108	Diarrhœa	Do.	Do.
	Control buff. calf	173	Temperature, Vesicles and Diarrhœa.	Do.	P. M. lesions : Typical of Rinderpest. Very severe reaction and worst lesions.
111	Goat	112	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	113	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	180	Do.	Do.	Do.
112	Goat	116	Temperature and Diarrhœa.	Died	P M. lesions : Typical of Rinderpest.
	Do.	117	Temperature	Do.	Do.
	Control buff. calf	188	Temperature, Vesicles and Diarrhœa.	Do.	Do.
113	Goat	121	Temperature and Diarrhœa.	Died	P. M. lesions : Typical of Rinderpest.
	Do.	122	Temperature	Do.	Do.
	Control buff. calf	196	Do.	Recovered.	

114	Goat . . .	125	Temperature . .	Died .	P. M. lesions : Typical of Rinderpest.
	Do. . .	126	Temperature and Diarrhœa.	Do. .	Do.
	Control buff. calf .	200	Temperature, Vesicles and Diarrhœa.	Do. .	Do.
115	Goat . .	129	Diarrhœa .	Died .	P. M. lesions : Typical of Rinderpest.
	Do. .	130	Temperature .	Do. .	Do.
	Control buff. calf .	207	Diarrhœa and Vesicles.	Recovered.	
116	Goat . .	134	Diarrhœa .	Died .	P. M. lesions : Typical of Rinderpest.
	Do. .	135	Temperature and Diarrhœa.	Do. .	Do.
	Control buff. calf .	214	Temperature and Vesicles.	Recovered.	
117	Goat . .	138	Temperature .	Died .	P. M. lesions : Typical of Rinderpest.
	Do. . .	139	Not pronounced .	Do. .	Do.
	Control buff. calf .	222	Non-reactor .	Recovered.	
118	Goat . .	142	Temperature and Diarrhœa.	Died .	P. M. lesions : Typical of Rinderpest.
	Do. . .	143	Not pronounced .	Do. .	Do.
	Control buff. calf .	227	Do. .	Recovered.	
119	Goat . .	146	Temperature and Diarrhœa.	Died .	P. M. lesions : Typical of Rinderpest.
	Do. . .	147	Not pronounced .	Do. .	Do.
	Control buff. calf .	235	Non-reactor .	Recovered.	

TABLE I—*contd.*

Passage Number	Kind of animal	No.	Reaction	Result	Remarks
120	Goat	150	Not pronounced	Died	P. M. lesions : Typical of Rinderpest.
	Do.	151	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	241	Non-reactor.		
121	Goat	154	Not pronounced	Died	P. M. lesions : Typical of Rinderpest.
	Do.	155	Temperature	Do.	Do.
	Control buff. calf	248	Vesicles	Recovered.	
122	Goat	158	Temperature and Diarrhœa.	Died	P. M. lesions : Typical of Rinderpest.
	Do.	159	Do.	Do.	Do.
	Control buff. calf	256	Non-reactor.		
123	Goat	163	Not pronounced	Died	P. M. lesions : Typical of Rinderpest.
	Do.	164	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	260	Non-reactor.		
124	Goat	167	Not pronounced	Died	P. M. lesions : Typical of Rinderpest.
	Do.	168	Temperature	Do.	Do.
	Control buff. calf	264	Temperature and Vesicles.	Recovered.	
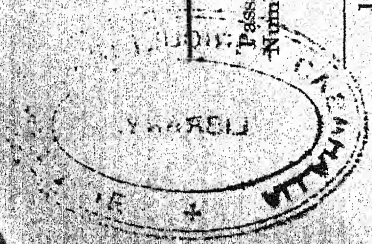
125	Goat	171	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	172	Not pronounced	Do.	Do.
	Control buff. calf	269	Do.	Recovered.	
126	Goat	176	Not pronounced	Died	P. M. lesions : Typical of Rinderpest.
	Do.	177	Temperature	Do.	Do.
	Control buff. calf	274	Vesicles	Recovered.	
127	Goat	182	Not pronounced	Died	P. M. lesions : Typical of Rinderpest.
	Do.	183	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	280	Temperature, Vesicles and Diarrhœa.	Recovered.	
128	Goat	190	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	191	Do.	Do.	Do.
	Control buff. calf	285	Temperature and Diarrhœa.	Recovered.	
129	Goat	195	Temperature and Diarrhœa.	Died	P. M. lesions : Typical of Rinderpest.
	Do.	196	Not pronounced	Do.	Do.
	Control buff. calf	290	Temperature, Vesicles and Diarrhœa.	Recovered.	
130	Goat	200	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	201	Diarrhœa	Do.	P. M. lesions : Typical of Rinderpest with pneumonia.
	Control buff. calf	295	Non-reactor.		

TABLE I—*contd.*

Passage Number	Kind of animal	No.	Reaction	Result	Remarks
131	Goat	204	Diarrhœa	Died	P. M. lesions : Typical of Rinderpest.
	Do.	205	Not pronounced	Do.	Do.
	Control buff. calf	301	Temperature, Vesicles and Diarrhœa.	Recovered.	
132	Goat	210	Temperature	Died.	
	Do.	211	Do.	Do.	
	Control buff. calf	305	Vesicles and Diarrhœa.	Recovered.	
133	Goat	214	Temperature and Diarrhœa.	Died	P. M. lesions : Typical of Rinderpest.
	Do.	215	Not pronounced	Do.	Do.
	Control buff. calf	311	Temperature, Vesicles and Diarrhœa.	Do.	Do.
134	Goat	218	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	219	Do.	Do.	Do.
	Control buff. calf	315	Temperature, Vesicles and Diarrhœa.	Recovered.	
135	Goat	222	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	223	Diarrhœa	Do.	Do.
	Control buff. calf	320	Temperature	Recovered.	

	Animal	No.	Symptoms	Result	P. M. lesions
136	Goat	227	Temperature and Diarrhœa.	Died	P. M. lesions : Typical of Rinderpest. (Abortion).
	Do.	228	Temperature	Recovered	
	Control buff. calf	324	Temperature and Vesicles.	Do.	
137	Goat	231	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	232	Do.	Do.	Do. (with pneumonia.)
	Control buff. calf	331	Non-reactor	.	
138	Goat	235	Temperature and Diarrhœa.	Died	P. M. lesions : Typical of Rinderpest.
	Do.	236	Do.	Do.	Do.
	Control buff. calf	3	Do.	Recovered.	
139	Goat	4	Not pronounced	Died	P. M. lesions : Typical of Rinderpest.
	Do.	5	Temperature	Do.	Do.
	Control buff. calf	10	Not pronounced	Recovered.	
140	Goat	9	Diarrhœa	Died	P. M. lesions : Typical of Rinderpest.
	Do.	11	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	17	Temperature, Vesicles and Diarrhœa.	Recovered.	
141	Goat	13	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	14	Temperature and Diarrhœa.	Do.	Do. (with pneumonia.)
	Control buff. calf	25	Temperature, Vesicles and Diarrhœa.	Do.	P. M. lesions : Typical of Rinderpest.

TABLE I—*contd.*

Passage Number	Kind of animal	No.	Reaction	Result	Remarks
142	Goat	17	Temperature and Diarrhœa.	Died	P. M. lesions : Typical of Rinderpest with pneumonia.
	Do.	18	Temperature	Do.	P. M. lesions : Typical of Rinderpest.
	Control buff. calf.	32	Temperature and Diarrhœa.	Recovered.	
143	Goat	21	Temperature and Vesicles.	Died	P. M. lesions : Typical of Rinderpest.
	Do.	22	Temperature and Diarrhœa.	Recovered.	
	Control buff. calf	38	Do.	Do.	
144	Goat	25	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	26	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	46	Temperature, Vesicles and Diarrhœa.	Do.	Do.
145	Goat	29	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	30	Diarrhœa	Do.	Do. (with pneumonia.)
	Control buff. calf	53	Temperature, Vesicles and Diarrhœa.	Do.	P. M. lesions : Typical of Rinderpest.
146	Goat	34	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	35	Do.	Do.	Do.
	Control buff. calf	60	Vesicles and Diarrhœa	Recovered.	

147	Goat	40	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	41	Do.	Do.	Do.
	Control buff. calf	68	Do.	Do.	Do.
148	Goat	44	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	45	Do.	Do.	Do. (with pneumonia.)
	Control buff. calf	73	Temperature, Vesicles and Diarrhœa.	Recovered.	
149	Goat	51	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	52	Temperature and Diarrhœa.	Do.	Do.
	Control buff. calf	78	Non-reactor.		
150	Goat	55	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	56	Do.	Do.	Do.
	Control buff. calf	83	Temperature and Diarrhœa.	Recovered.	

TABLE II

Muktesar Strain

Passage Number	Kind of animal	No. of the animal	Reaction	Result	Remarks
1	Goat	53	Temperature and Diarrhœa.	Died	P. M. lesions: Typical of Rinderpest.
	Do.	54	Temperature, Diarrhœa and Vesicles.	Do.	Do.
2	Goat	57	Temperature	Recovered.	
	Do.	58	Do.	Died	P. M. lesions: Typical of Rinderpest.
3	Goat	61	Temperature and Diarrhœa.	Died	P. M. lesions: Typical of Rinderpest.
	Do.	62	Do.	Do.	Do.
4	Goat	65	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	66	Temperature and Diarrhœa.	Recovered	
5	Goat	69	Temperature, Vesicles and Diarrhœa.	Died	P. M. lesions: Typical of Rinderpest.
	Do.	70	Temperature	Do.	Do. with pneumonia.
6	Goat	73	Temperature and Diarrhœa.	Died	P. M. lesions : Typical of Rinderpest.
	Do.	74	Do.	Do.	Do.

7	Goat	77	Diarrhœa	Died	P. M. lesions : Typical of Rinderpest.
	Do.	78	Not pronounced	Do.	Do.
8	Goat	81	Temperature and Diarrhœa.	Died	P. M. lesions : Typical of Rinderpest.
	Do.	82	Do.	Do.	Do.
9	Goat	85	Temperature and Diarrhœa.	Died	P. M. lesions : Typical of Rinderpest.
	Do.	86	Not pronounced	Do.	Do.
10	Goat	89	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	90	Do.	Do.	Do.
11	Goat	93	Temperature and Diarrhœa.	Died	P. M. lesions, Typical of Rinderpest.
	Do.	94	Not pronounced	Do.	Do.
12	Goat	97	Temperature, Diarrhœa and Vesicles.	Died	P. M. lesions : Typical of Rinderpest.
	Do.	98	Not pronounced	Do.	Do.
13	Goat	101	Not pronounced	Died	P. M. lesions : Typical of Rinderpest.
	Do.	102	Temperature	Do.	Do.
14	Goat	107	Not pronounced	Died	P. M. lesions : Typical of Rinderpest.
	Do.	108	Do.	Do.	Do.
15	Goat	113	Temperature, Vesicles and Diarrhœa.	Died	P. M. lesions : Typical of Rinderpest.
	Do.	114	Temperature	Do.	Do.

TABLE II—contd.

Passage Number	Kind of animal	No. of the animal	Reaction	Result	Remarks
16	Goat	115	Temperature	Died	P. M. : Typical of Rinderpest.
	Do.	116	Not pronounced	Do.	Do.
17	Goat	119	Temperature, Vesicles and Diarrhœa.	Died	P. M. : Typical of Rinderpest.
	Do.	120	Do.	Do.	Do.
18	Goat	123	Temperature and Diarrhœa.	Died	P. M. : Typical of Rinderpest.
	Do.	124	Temperature	Do.	Do.
19	Goat	127	Temperature and Diarrhœa.	Died	P. M. : Typical of Rinderpest.
	Do.	128	Do.	Do.	Do. with pneumonia.
20	Goat	133	Temperature	Died	P. M. : Typical of Rinderpest.
	Do.	134	Temperature and Diarrhœa.	Do.	Do.
21	Goat	135	Temperature	Died	P. M. : Typical of Rinderpest.
	Do.	136	Not pronounced	Do.	
22	Goat	140	Temperature	Died	P. M. : Typical of Rinderpest with pneumonia.
	Do.	141	Not pronounced	Do.	P. M. : Typical of Rinderpest.
23	Goat	146	Temperature	Died	P. M. : Typical of Rinderpest.
	Do.	147	Diarrhœa	Do.	Do.

24	Goat	152	Not pronounced	Died	P. M. : Typical of Rinderpest.
	Do.	153	Do.	Do.	Do.
25	Goat	154	Diarrhœa	Died	P. M. : Typical of Rinderpest.
	Do.	155	Do.	Do.	Do.
26	Goat	158	Vesicles	Died	P. M. : Typical of Rinderpest.
	Do.	159	Diarrhœa	Do.	Do.
27	Goat	162	Temperature and Diarrhœa.	Died	P. M. : Typical of Rinderpest with pneumonia.
	Do.	163	Diarrhœa	Do.	
28	Goat	166	Not pronounced	Died	P. M. : Typical of Rinderpest.
	Do.	167	Do.	Do.	Do.
29	Goat	170	Diarrhœa	Died	P. M. : Typical of Rinderpest.
	Do.	171	Not pronounced	Do.	Do.
30	Goat	174	Temperature	Died	P. M. : Typical of Rinderpest.
	Do.	175	Not pronounced	Do.	Do.
31	Goat	1	Temperature	Died	P. M. : Typical of Rinderpest.
	Do.	2	Temperature and Diarrhœa.	Do.	Do.
32	Goat	5	Diarrhœa	Died	P. M. : Typical of Rinderpest.
	Do.	6	Temperature and Diarrhœa.	Do.	Do.

TABLE II—contd.

Passage Number	Kind of animal	No. of the animal	Reaction	Result	Remarks
33	Goat	9	Temperature and Diarrhœa.	Died	P. M. : Typical of Rinderpest.
	Do.	10	Diarrhœa	Do.	
34	Goat	13	Not pronounced.	Died	P. M. : Typical of Rinderpest.
	Do.	14	Temperature and Diarrhœa.	Do.	
35	Goat	17	Not pronounced.	Died	P. M. : Typical of Rinderpest.
	Do.	18	Diarrhœa	Do.	
36	Goat	22	Diarrhœa	Died	P. M. : Typical of Rinderpest.
	Do.	24	Temperature and Diarrhœa.	Do.	
37	Goat	25	Temperature and Diarrhœa	Died	P. M. : Typical of Rinderpest.
	Do.	26	Diarrhœa	Do.	
38	Goat	29	Temperature	Recovered	P. M. : Typical of Rinderpest.
	Do.	30	Temperature and Diarrhœa.	Died	

39	Goat	33	Temperature and Diarrhœa.	Died	P. M. : Typical of Rinderpest.
	Do.	34	Not pronounced.	Do.	Do. (with pneumonia).
40	Goat	37	Diarrhœa	Recovered	P. M. : Typical of Rinderpest (with pneumonia).
	Do.	38	Temperature and Diarrhœa.	Died	
41	Goat	42	Temperature	Died	P. M. : Typical of Rinderpest.
	Do.	43	Temperature and Diarrhœa.	Do.	Do.
42	Goat	46	Not pronounced.	Died	P. M. : Typical of Rinderpest (with pneumonia).
	Do.	47	Do.	Do.	Do.
43	Goat	51	Temperature and Diarrhœa.	Died	P. M. : Typical of Rinderpest.
	Do.	52	Do.	Do.	Do.
44	Goat	55	Temperature and Diarrhœa.	Died	P. M. : Typical of Rinderpest (with pneumonia).
	Do.	56	Not pronounced.	Do.	P. M. : Typical of Rinderpest.
45	Goat	59	Temperature	Recovered	
	Do.	60	Not pronounced.	Died	P. M. : Typical of Rinderpest.
46	Goat	63	Not pronounced	Recovered	
	Do.	64	Temperature and Diarrhœa.	Died	P. M. lesions : Typical of Rinderpest (complicated with pneumonia.)
47	Goat	67	Not pronounced.	Died	P. M. : Typical of Rinderpest.
	Do.	68	Do.	Do.	P. M. lesions : Typical of Rinderpest.

TABLE II—contd.

Passage Number	Kind of animal	No. of the animal	Reaction	Result	Remarks
48	Goat	71	Temperature .	Died . .	P. M. lesions: Typical of Rinderpest.
	Do.	72	Temperature and Diarrhœa.	Do. . .	Do.
49	Goat	75	Temperature and Diarrhœa.	Died . .	P. M. lesions: Typical of Rinderpest.
	Do.	76	Do. .	Do. .	Do.
50	Goat	80	Not pronounced.	Died .	P. M. lesions: Typical of Rinderpest.
	Do.	81	Temperature and Diarrhœa.	Do. .	Do.
51	Goat	84	Diarrhœa .	Died .	P. M. lesions: Typical of Rinderpest.
	Do.	85	Not pronounced.	Do. .	Do.
52	Goat	89	Temperature .	Died .	P. M. lesions: Typical of Rinderpest.
	Do.	90	Temperature and Diarrhœa.	Do. .	Do.
53	Goat	94	Diarrhœa .	Died .	P. M. lesions: Typical of Rinderpest.
	Do.	95	Temperature .	Do. .	Do.
54	Goat	98	Temperature .	Died .	P. M. lesions: Typical of Rinderpest.
	Do.	99	Temperature and Diarrhœa.	Do. .	Do.
55	Goat	105	Diarrhœa and	Died .	P. M. lesions: Typical of Rinderpest.
	Do.	106	Temperature Diarrhœa.	Do.	Do.

56	Goat	.	.	.	109	Temperature	.	Died	.	P. M. lesions : Typical of Rinderpest.
	Do.	.	.	.	110	Do.	.	Do.	.	Do.
57	Goat	.	.	.	114	Temperature	.	Died	.	P. M. lesions : Typical of Rinderpest.
	Do.	.	.	.	115	Not pronounced.	.	Do.	.	Do.
58	Goat	.	.	.	118	Temperature	.	Died	.	P. M. lesions : Typical of Rinderpest.
	Do.	.	.	.	120	Do.	.	Do.	.	Do.
59	Goat	.	.	.	123	Temperature	.	Died	.	P. M. lesions : Typical of Rinderpest.
	Do.	.	.	.	124	Do.	.	Do.	.	Do.
60	Goat	.	.	.	127	Temperature and Diarrhœa.	.	Died	.	P. M. lesions : Typical of Rinderpest.
	Do.	.	.	.	128	Do.	.	Do.	.	Do.
61	Goat	.	.	.	131	Not pronounced.	.	Died	.	P. M. lesions : Typical of Rinderpest.
	Do.	.	.	.	133	Temperature and Diarrhœa.	.	Do.	.	Do.
62	Goat	.	.	.	136	Not pronounced.	.	Died	.	P. M. lesions : Typical of Rinderpest.
	Do.	.	.	.	137	Temperature and Diarrhœa.	.	Do.	.	Do.
63	Goat	.	.	.	140	Not pronounced.	.	Died	.	P. M. lesions : Typical of Rinderpest.
	Do.	.	.	.	141	Temperature and Diarrhœa.	.	Do.	.	Do.
64	Goat	.	.	.	144	Not pronounced	.	Died	.	P. M. lesions : Typical of Rinderpest.
	Do.	.	.	.	145	Temperature	.	Do.	.	Do.

TABLE II—cont'l.

Passage Number	Kind of animal	No. of the animal	Reaction	Result	Remarks
65	Goat	148	Temperature and Diarrhœa.	Died	P.M. lesions : Typical of Rinderpest.
	Do.	149	Not pronounced.	Do.	Do.
66	Goat	152	Diarrhœa	Died	P.M lesions : Typical of Rinderpest.
	Do.	153	Not pronounced.	Do.	Do.
67	Goat	156	Temperature	Died	P.M. lesions : Typical of Rinderpest.
	Do.	157	Not pronounced.	Do.	Do.
68	Goat	161	Temperature	Died	P.M. lesions : Typical of Rinderpest.
	Do.	162	Do.	Do.	Do.
69	Goat	165	Not pronounced.	Died	P.M. lesions : Typical of Rinderpest.
	Do.	166	Diarrhœa	Do.	Do.
70	Goat	169	Temperature and Diarrhœa.	Died	P.M. lesions : Typical of Rinderpest.
	Do.	170	Temperature	Do.	Do.
71	Goat	174	Diarrhœa	Died	P.M. lesions : Typical of Rinderpest.
	Do.	175	Not pronounced.	Do.	Do.
72	Goat	180	Temperature	Died	P.M. lesions : Typical of Rinderpest.
	Do.	181	Do.	Do.	Do.

		No.	Symptoms		Post-mortem
73	Goat	185	Temperature Diarrhoea.	and Died	P. M. lesions : Typical of Rinderpest.
	Do.	186	Temperature	Do.	Do.
74	Goat	193	Temperature Diarrhoea.	and Died	P. M. lesions : Typical of Rinderpest.
	Do.	194	Temperature Diarrhoea.	and Recovered	Do.
75	Goat	198	Temperature	Recovered	
	Do.	199	Temperature Diarrhoea.	and Died	P. M. lesions : Typical of Rinderpest.
76	Goat	202	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	203	Do.	Do.	Do.
77	Goat	208	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	209	Do.	Do.	Do.
78	Goat	212	Temperature Diarrhoea.	and Recovered	
	Do.	213	Do.	Died	P.M. lesions Typical of Rinderpest.
79	Goat	216	Temperature Diarrhoea.	and Died	P. M. lesions : Typical of Rinderpest and Diarrhœa
	Do.	217	Temperature	Do.	Do.
80	Goat	220	Temperature	Died	P. M. lesions : Typical of Rinderpest.
	Do.	221	Not pronounced.	Do.	Do. (with pneumonia).
81	Goat	225	Temperature	Died	P. M. lesions : Typical of Rinderpest
	Do.	226	Temperature Diarrhoea.	and Do.	P. M. lesions : Typical of Rinderpest (with pneumonia).

TABLE II—*contd.*

Passage Number	Kind of animal	No. of the animal	Reaction	Result	Remarks
82	Goat	229	Temperature	Died	P. M. lesions : Typical of Rinderpest (with pneumonia).
	Do.	230	Do.	Do.	P. M. lesions : Typical of Rinderpest.
83	Goat	233	Diarrhœa	Died	P. M. lesions : Typical of Rinderpest.
	Do.	234	Not pronounced.	Do.	Do.
84	Goat	2	Temperature and Diarrhœa.	Died	P. M. lesions Typical of Rinderpest.
	Do.	3	Do.	Do.	Do.
85	Goat	6	Temperature	Died	P. M. lesions : Typical of Rinderpest (with pneumonia).
	Do.	7	Temperature	Do.	Do.
86	Goat	10	Temperature	Died	P. M. lesions : Typical of Rinderpest (with pneumonia).
	Do.	12	Do.	Do.	Do.
87	Goat	15	Temperature	Died	P. M. lesions : Typical of Rinderpest (with pneumonia).
	Do.	16	Do.	Do.	Do.

88	Goat . .	.	.	.	19	Temperature . .	.	Died . .	P. M. lesions : Typical of Rinderpest.
	Do. .	.	.	.	20	Do. .	.	Do. .	Do.
89	Goat .	.	.	.	23	Temperature .	.	Died .	P. M. lesions : Typical of Rinderpest.
	Do. .	.	.	.	24	Temperature and Diarrhœa.	.	Do. .	Do.
90	Goat .	.	.	.	27	Temperature .	.	Died .	P. M. lesions : Typical of Rinderpest.
	Do. .	.	.	.	28	Temperature and Diarrhœa.	.	Recovered .	
91	Goat .	.	.	.	31	Temperature .	.	Died .	P. M. lesions : Typical of Rinderpest.
	Do. .	.	.	.	32	Temperature and Diarrhœa.	.	Do. .	Do.
92	Goat .	.	.	.	38	Not pronounced.	.	Died .	P. M. lesions : Typical of Rinderpest.
	Do. .	.	.	.	39	Temperature .	.	Do. .	Do.
93	Goat .	.	.	.	42	Temperature .	.	Died .	P. M. lesions : Typical of Rinderpest.
	Do. .	.	.	.	43	Temperature and Diarrhœa.	.	Do. .	Do.
94	Goat .	.	.	.	48	Temperature .	.	Died .	P. M. lesions : Typical of Rinderpest.
	Do. .	.	.	.	50	Do. .	.	Do. .	Do.
95	Goat .	.	.	.	53	Temperature .	.	Died .	P. M. lesions : Typical of Rinderpest.
	Do. .	.	.	.	54	Diarrhœa .	.	Recovered.	

AUGUST 1932.

Chart I.

CONTROL CALF № 136.
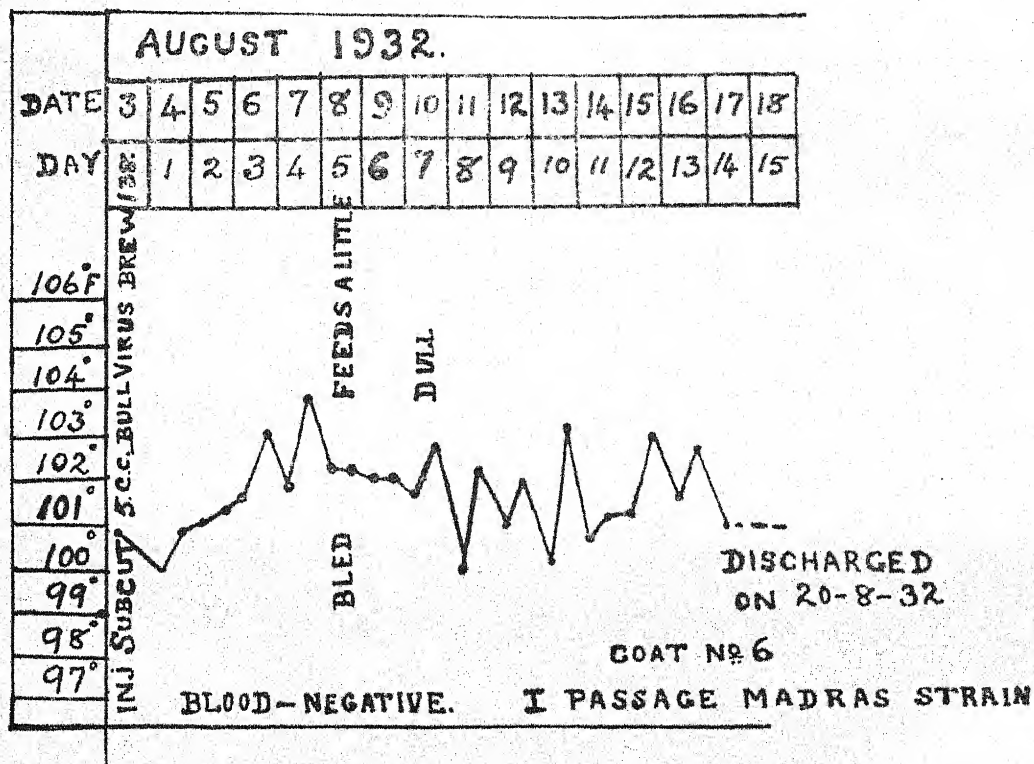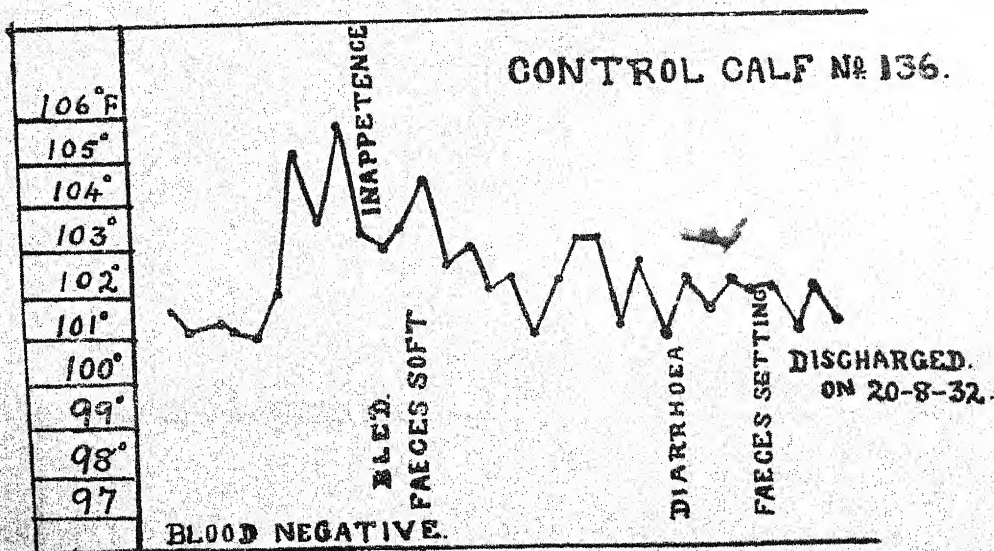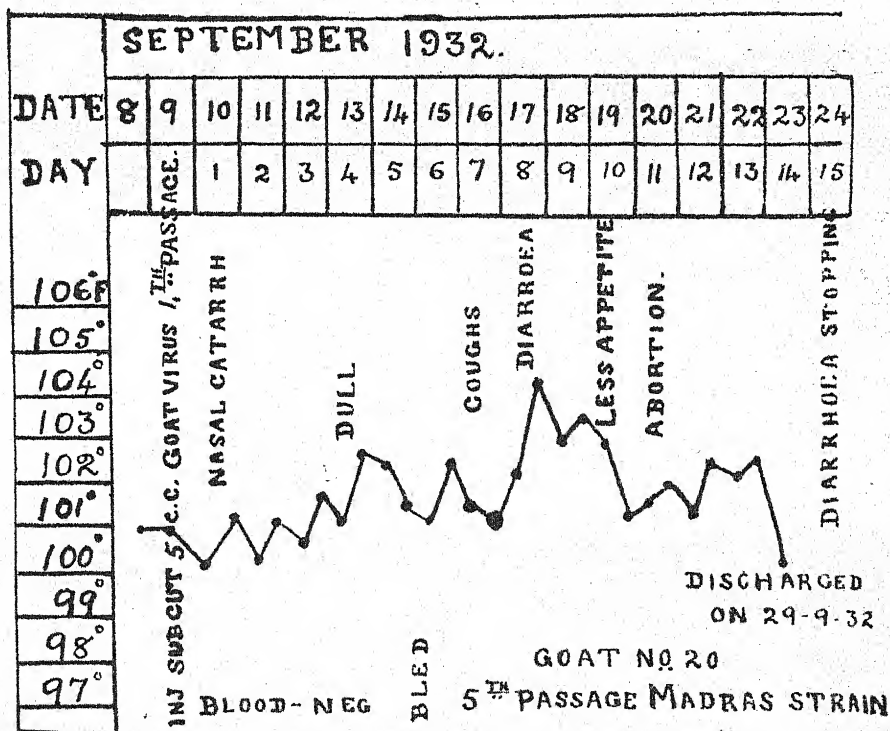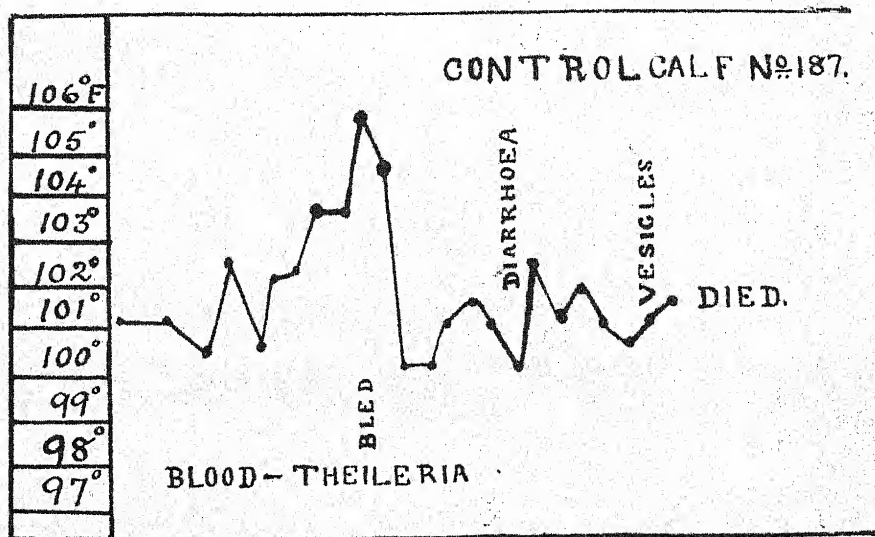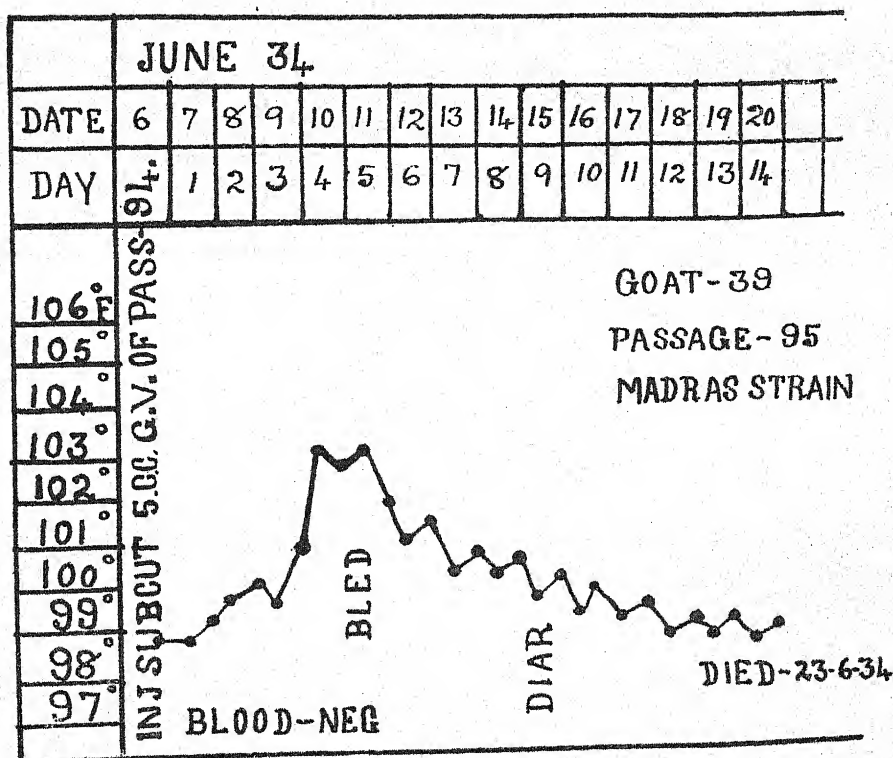
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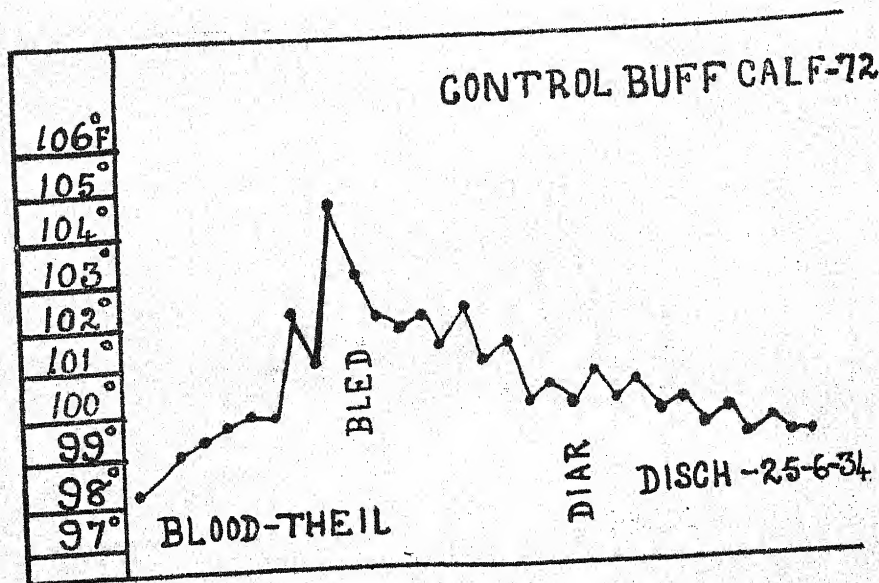
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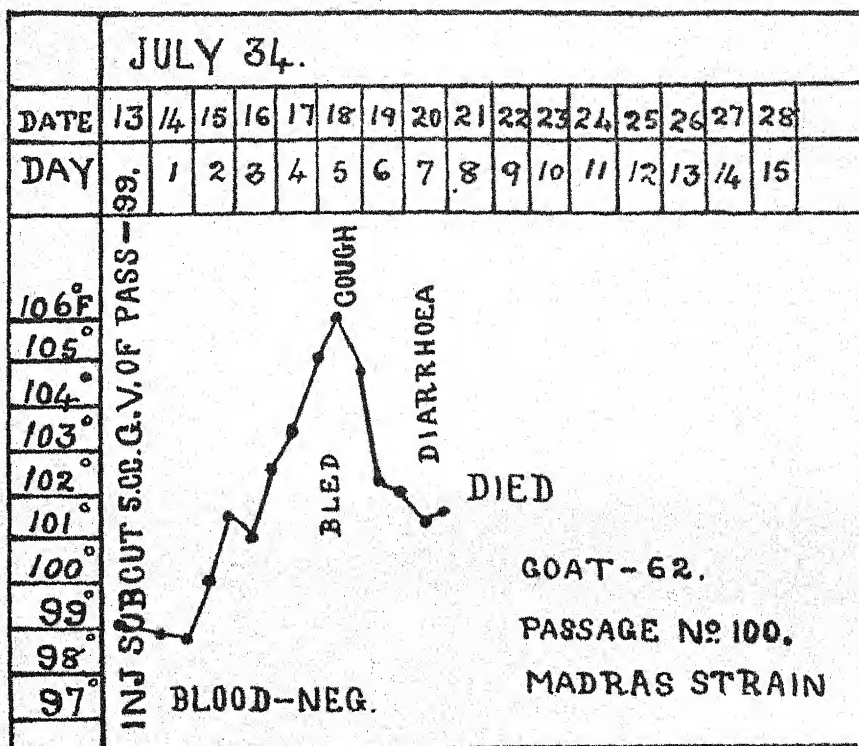

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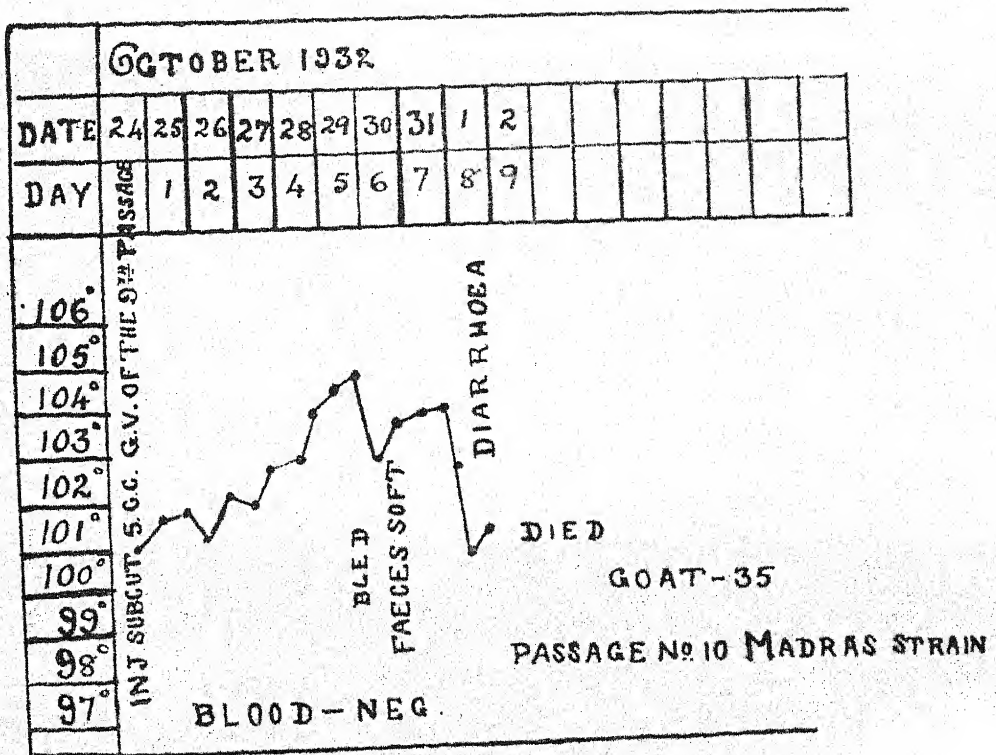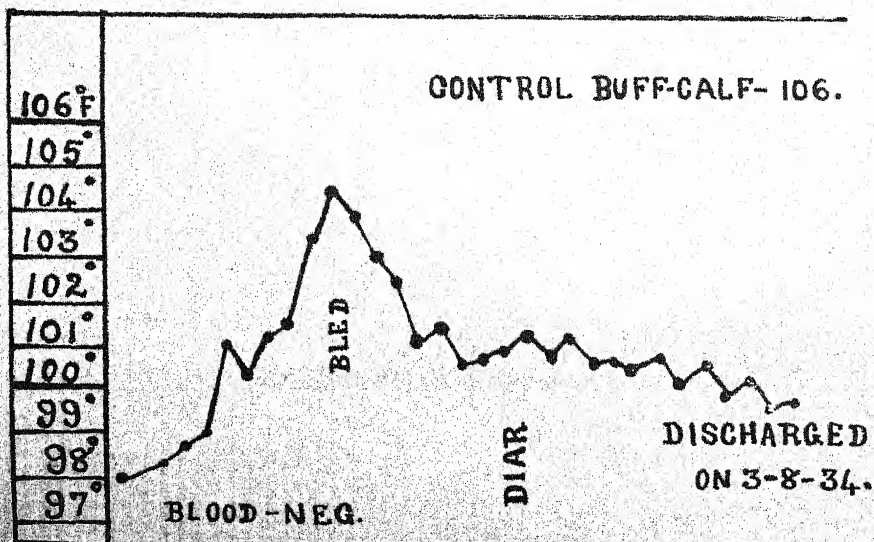
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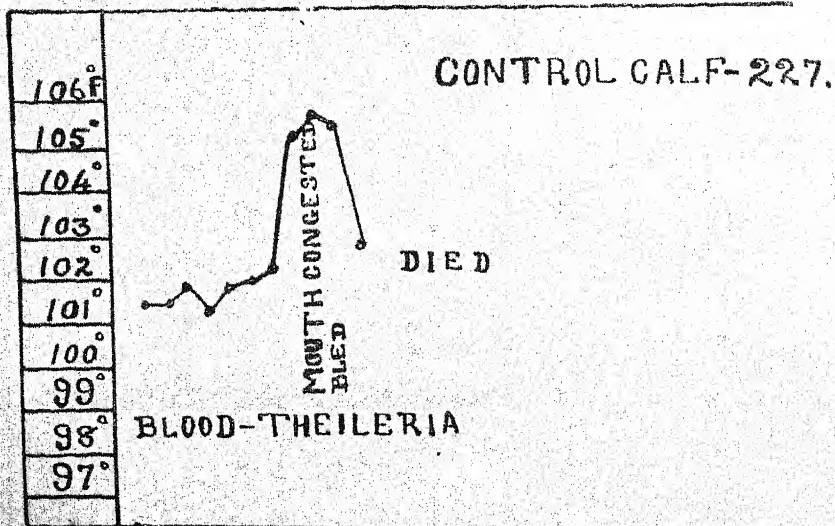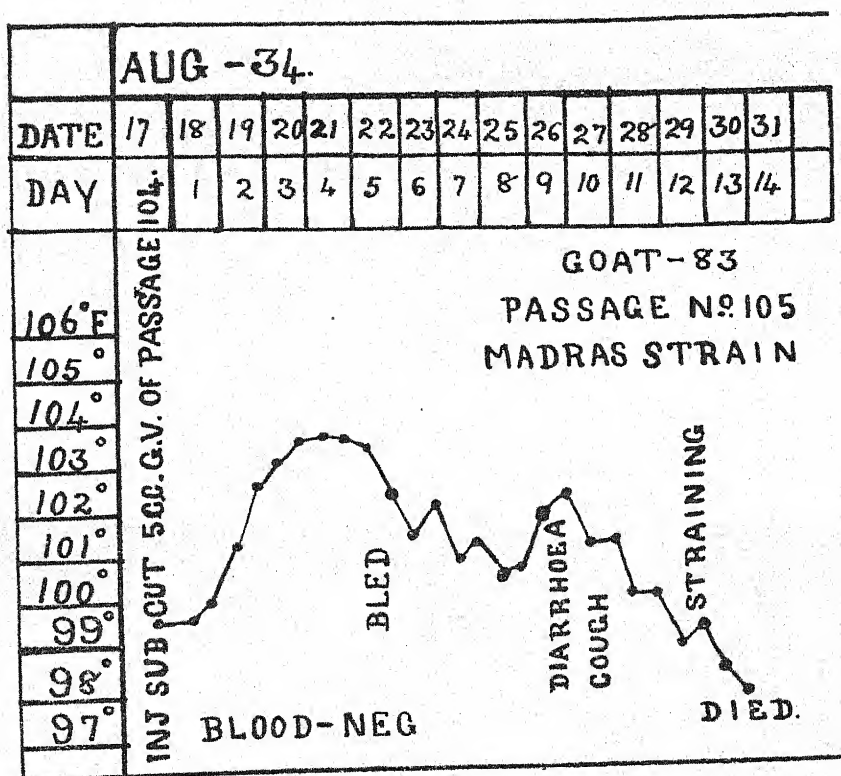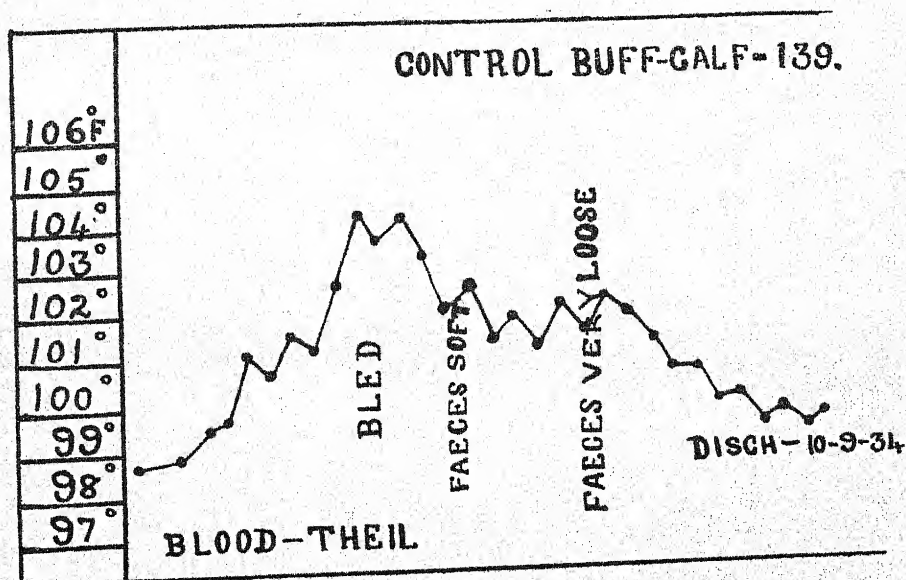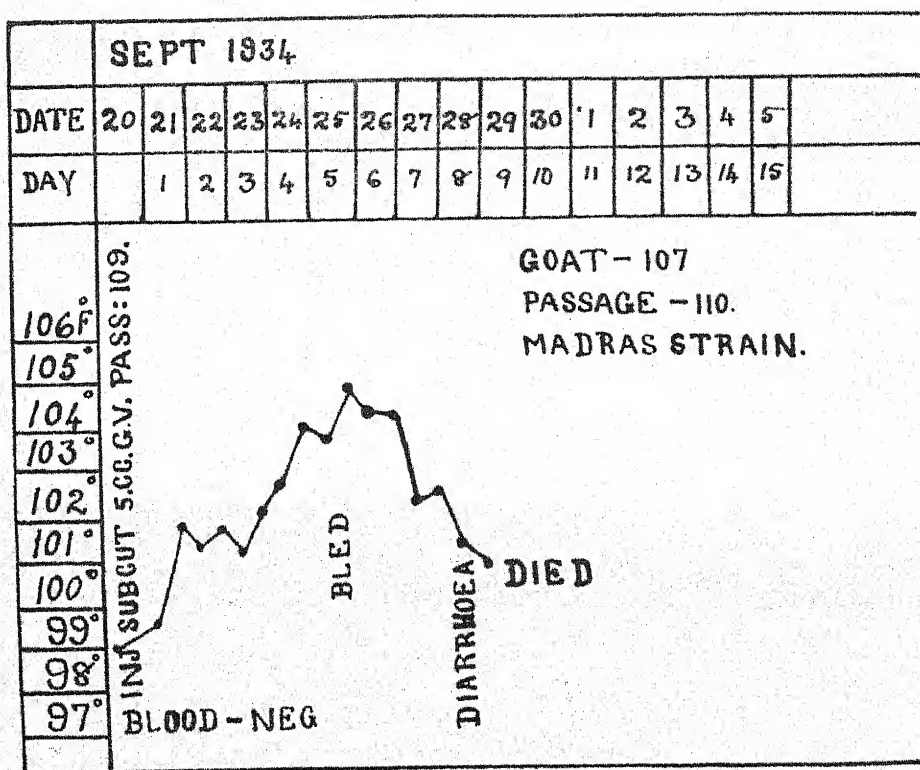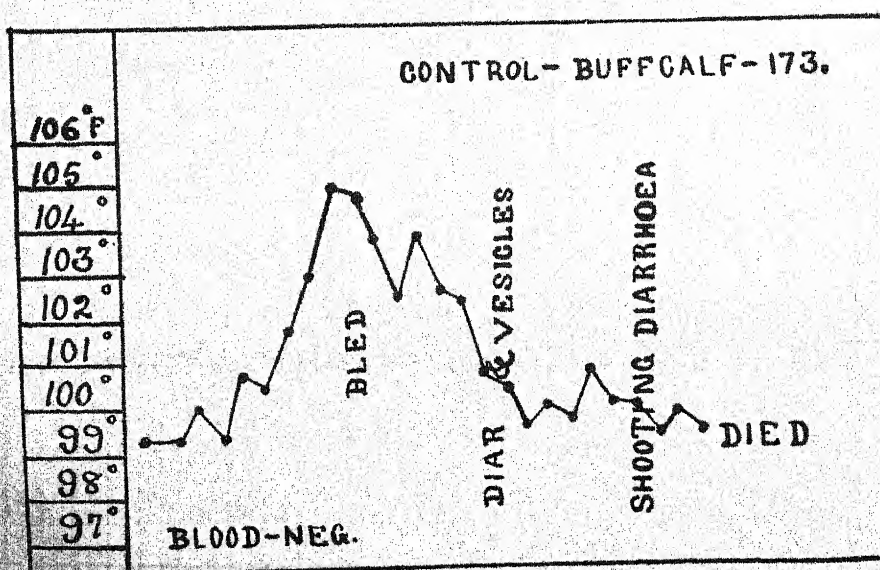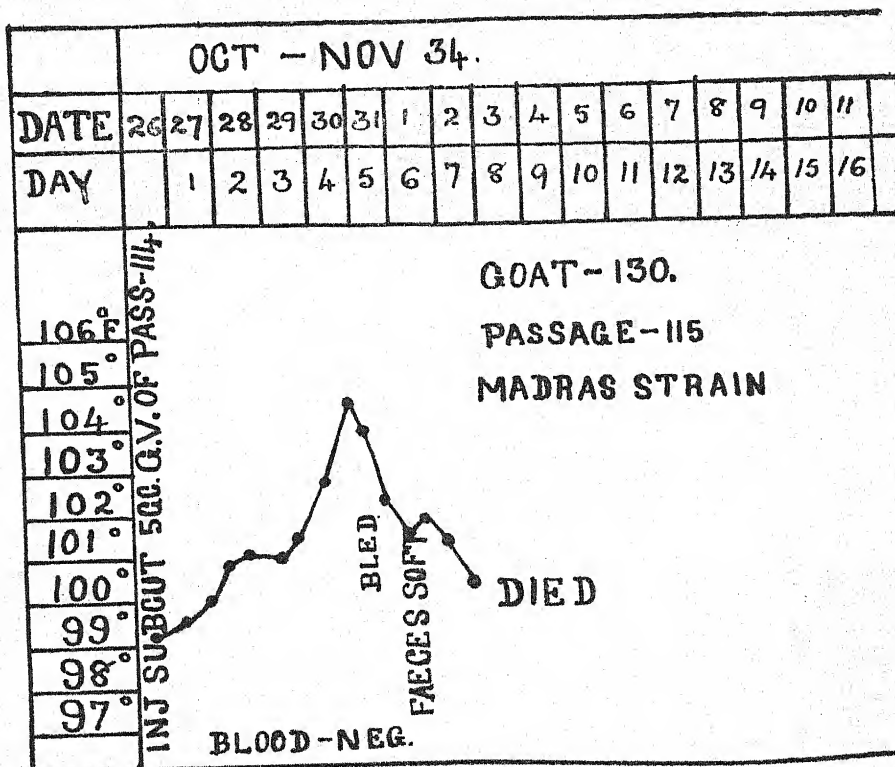

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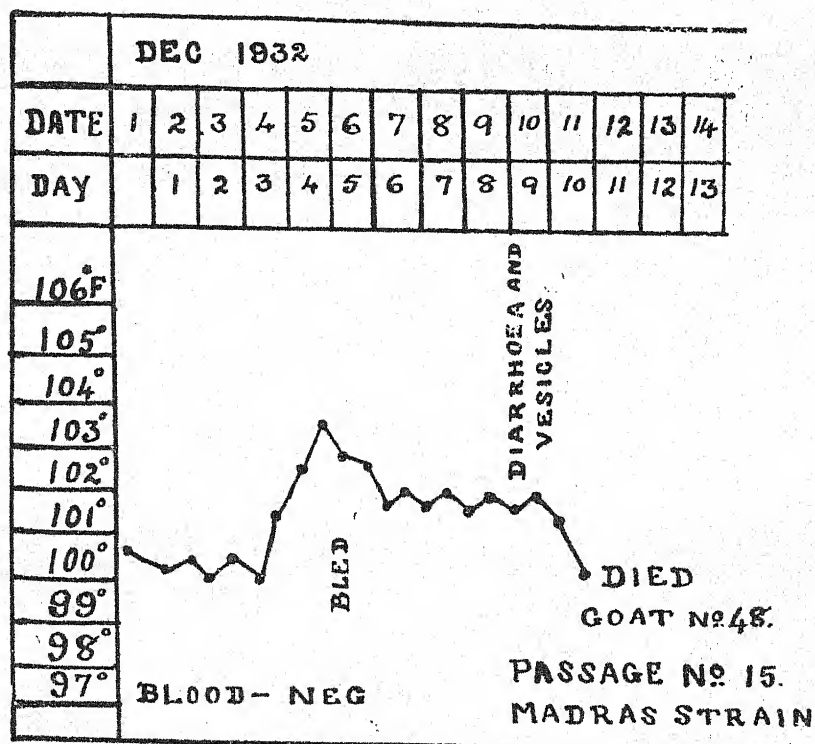
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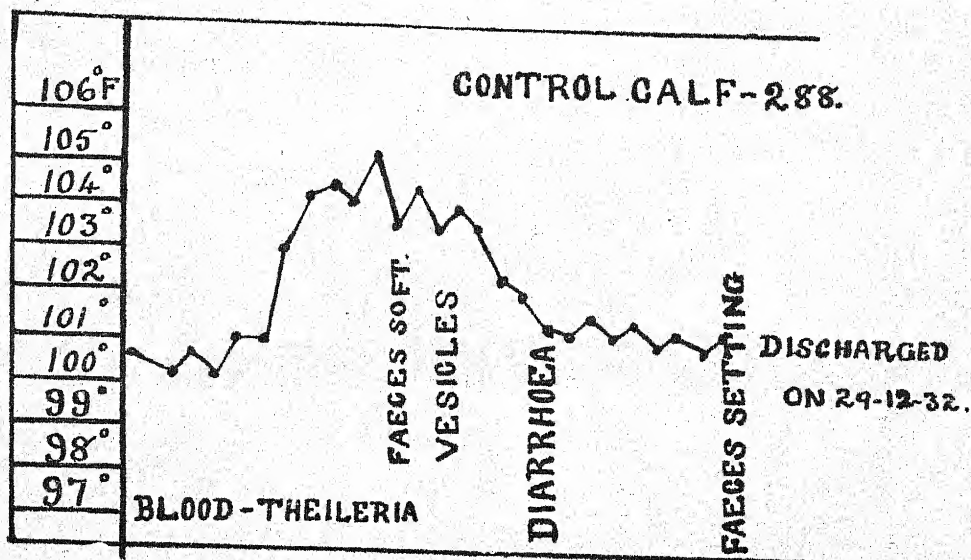
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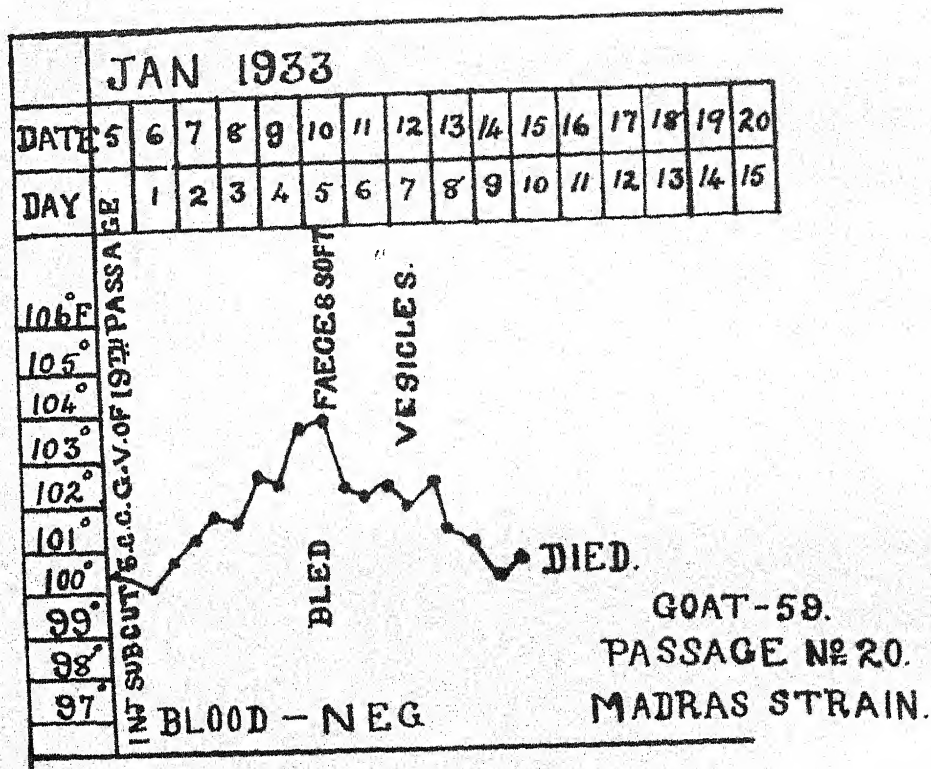
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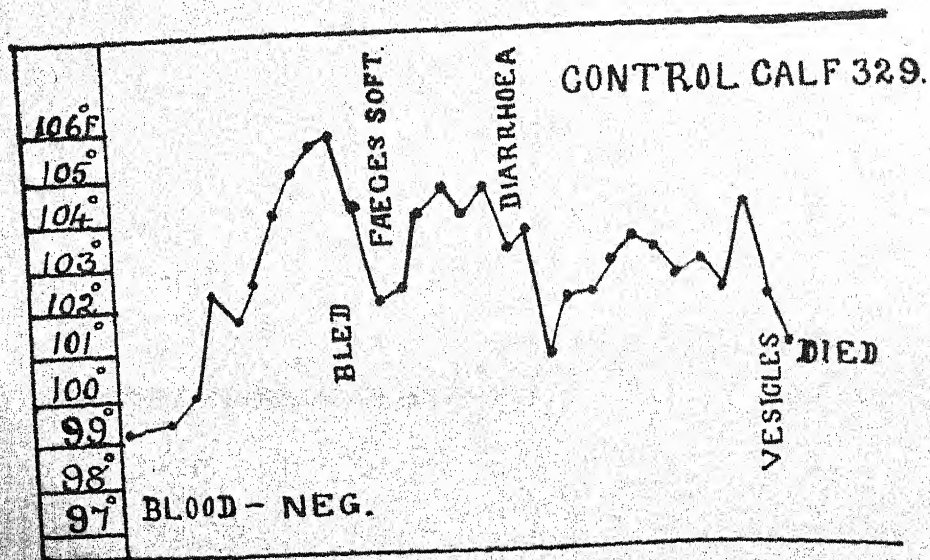
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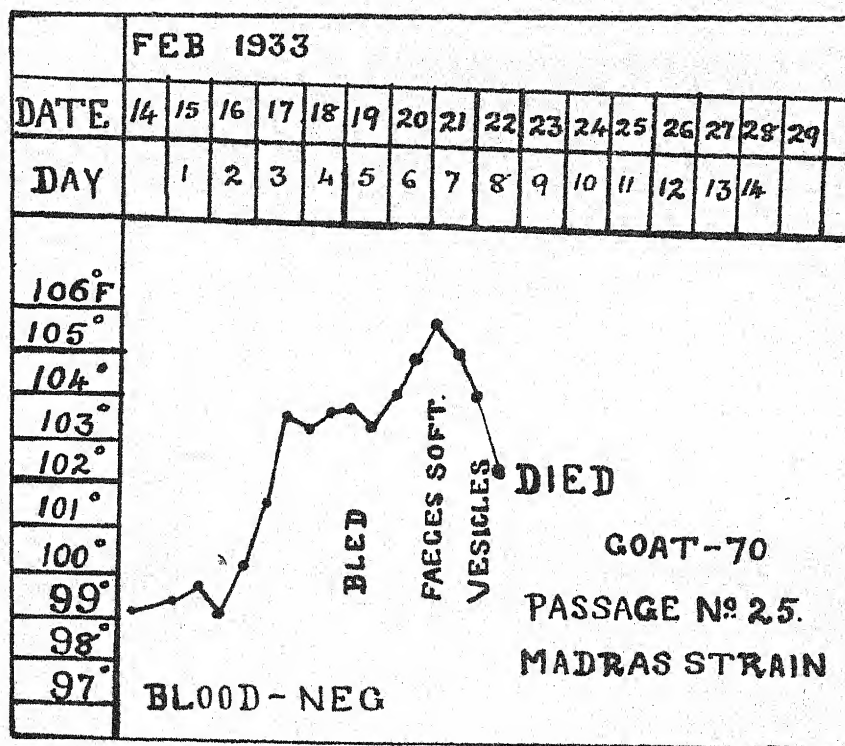
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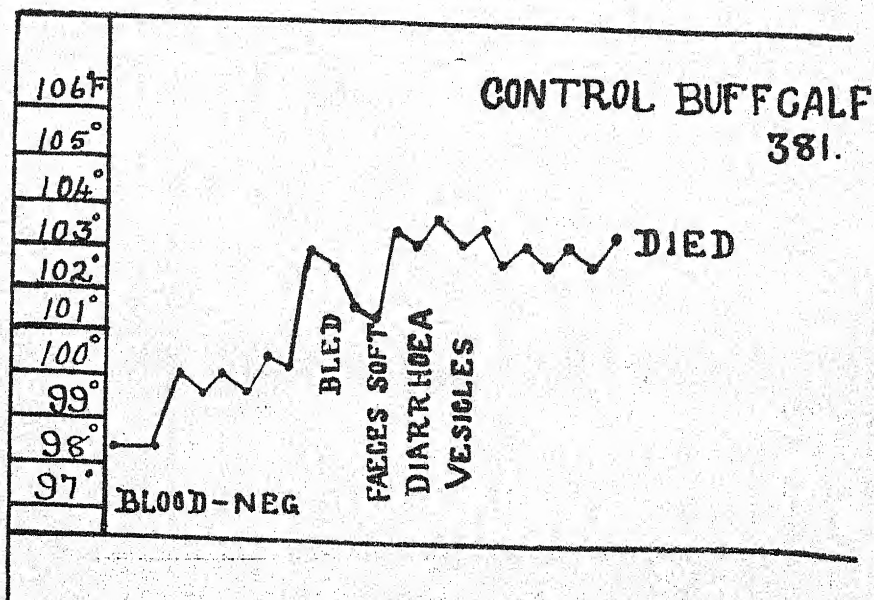
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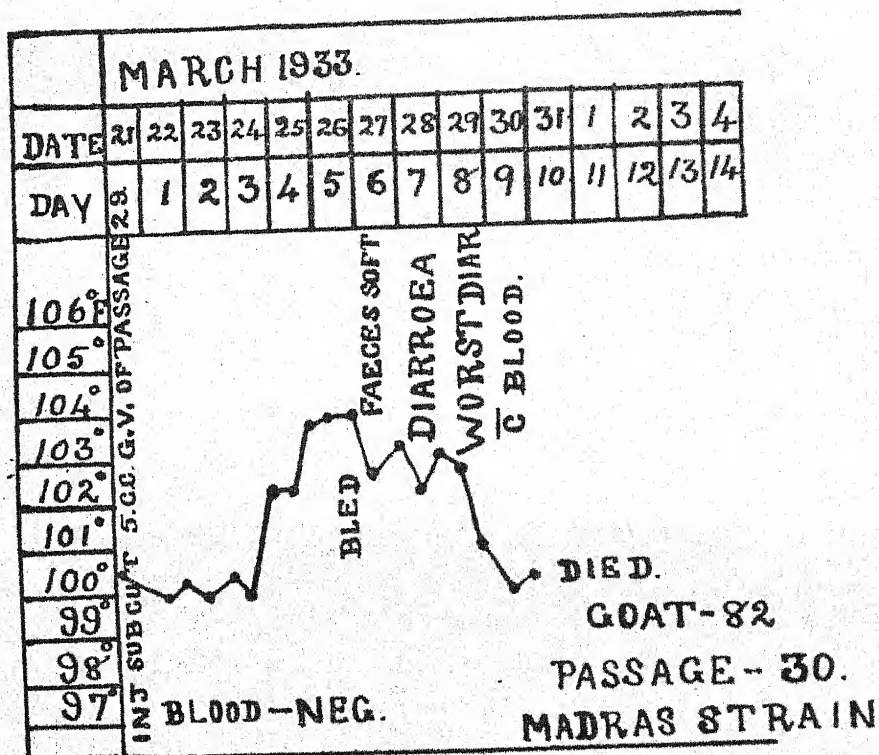
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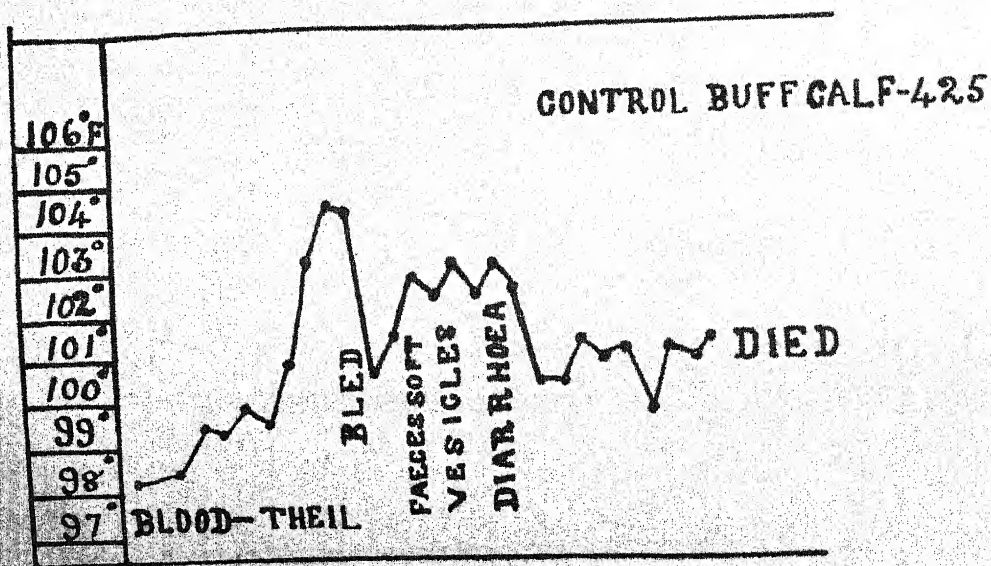
Chart XIV.

Chart XV.

Chart XVI.

JUNE 1933

DATE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
DAY		1	2	3	4	5	6	7	8	9	10	11	12	13	14

GOAT-21
PASSAGE Nº 40.
MADRAS STRAIN

BLED   COUGHS   FAECES SOFT   DIAR   VES   DIED

BLOOD —NEG

Chart XVII.

CONTROL BUFF
CALF-68

BLED   VES   DIAR   DISCHAGED
ON 24-6-33
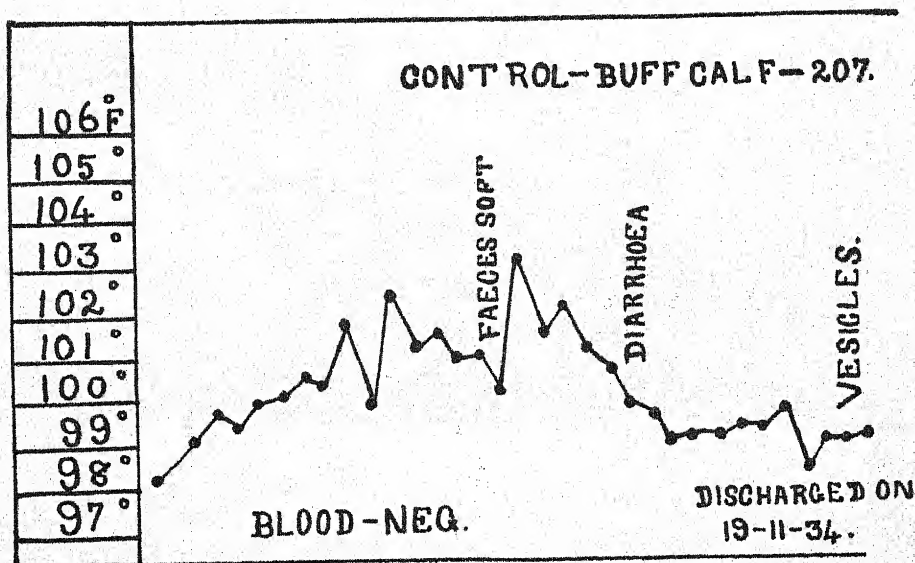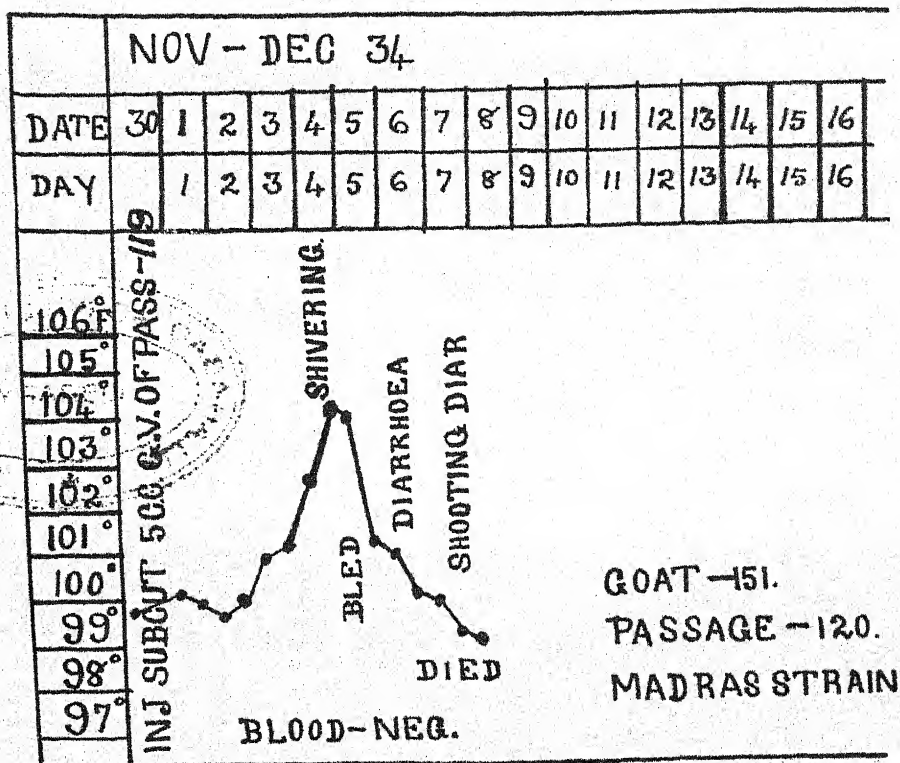
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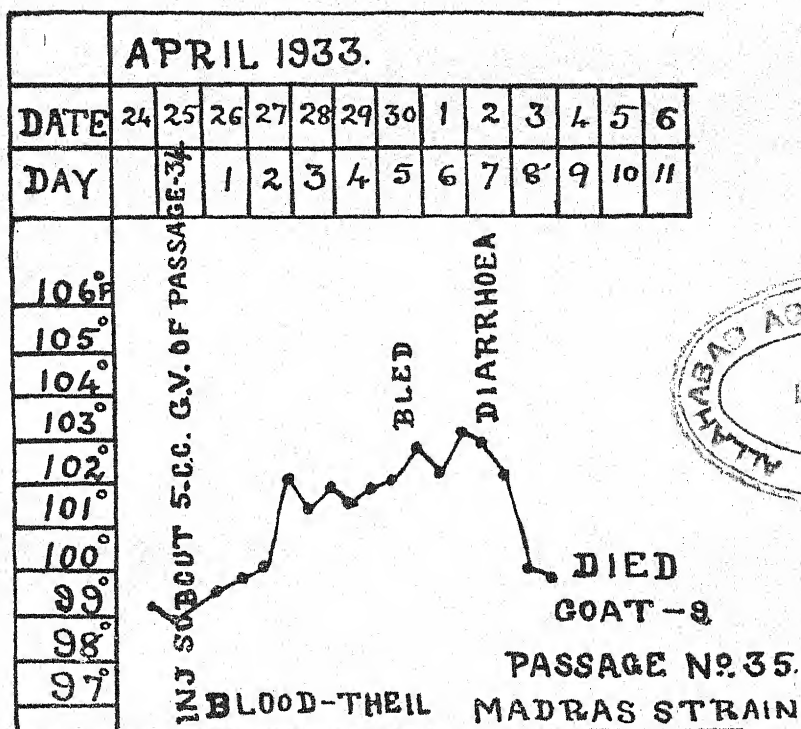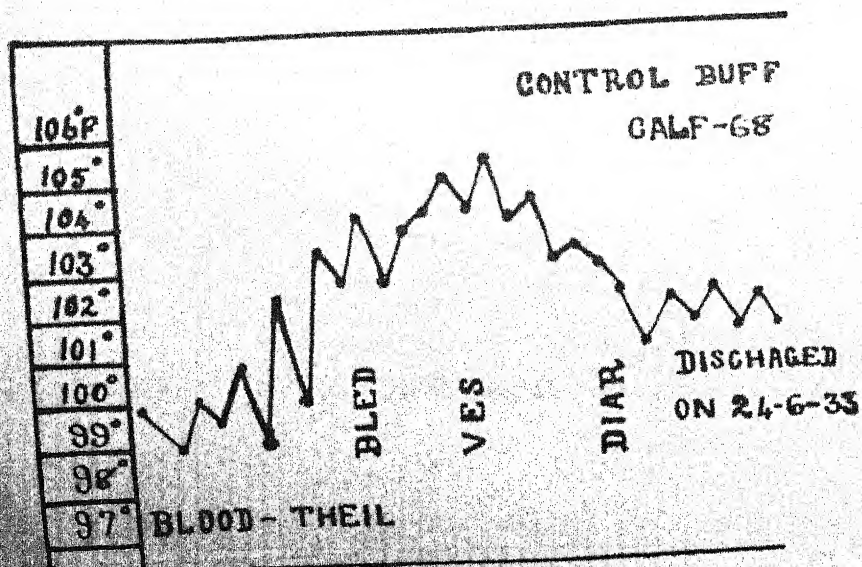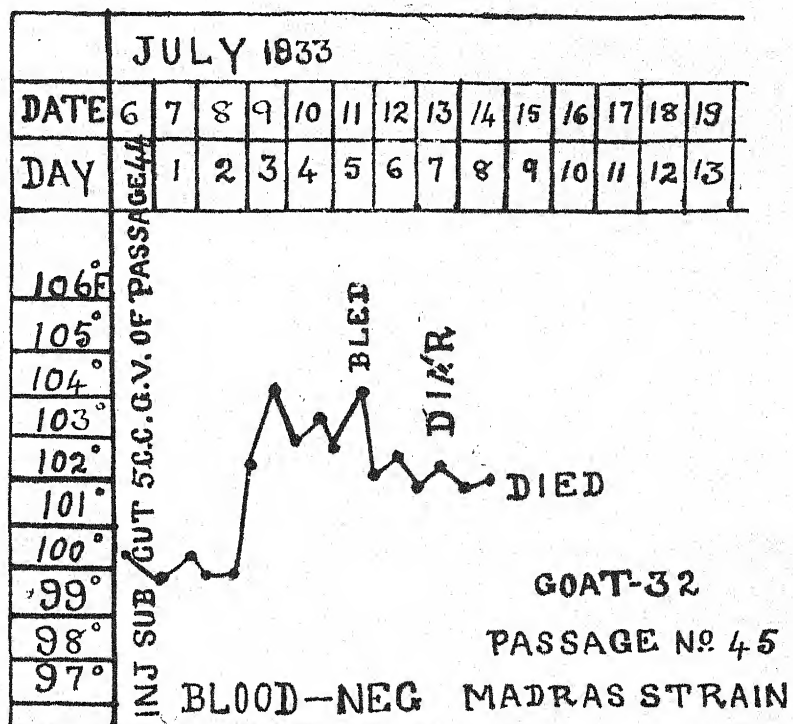
Chart XVIII.

Chart XIX.

Chart XX.

Chart XVII.

Chart XVIII.
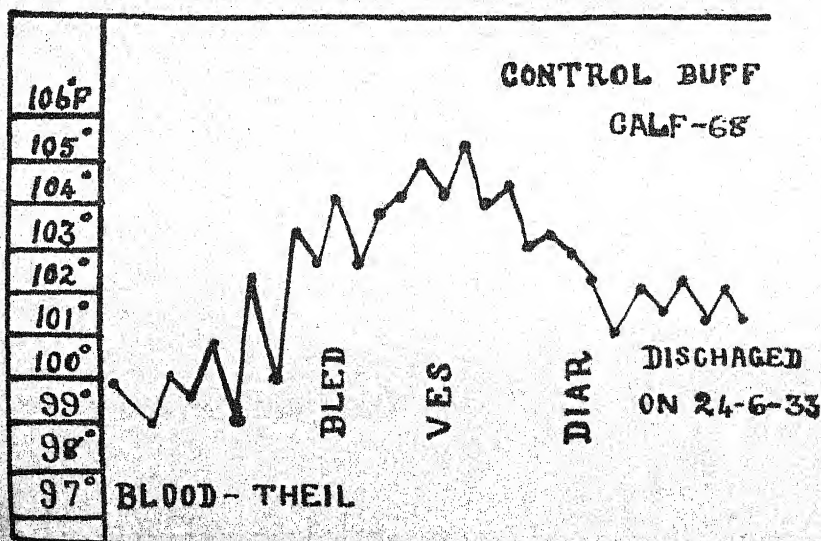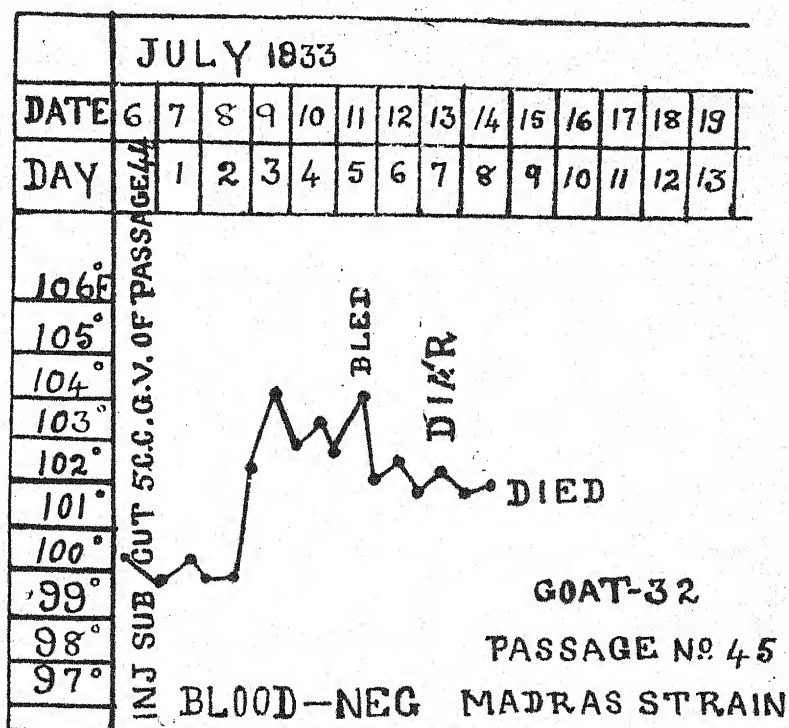
Chart XIX.
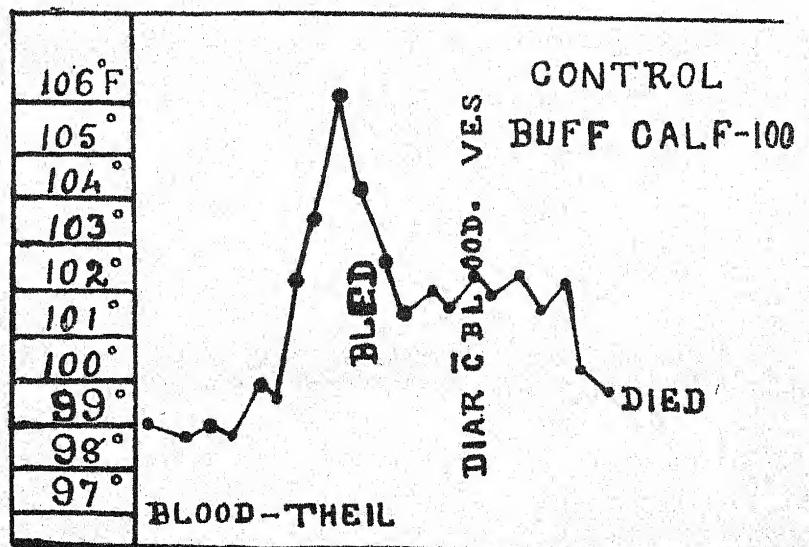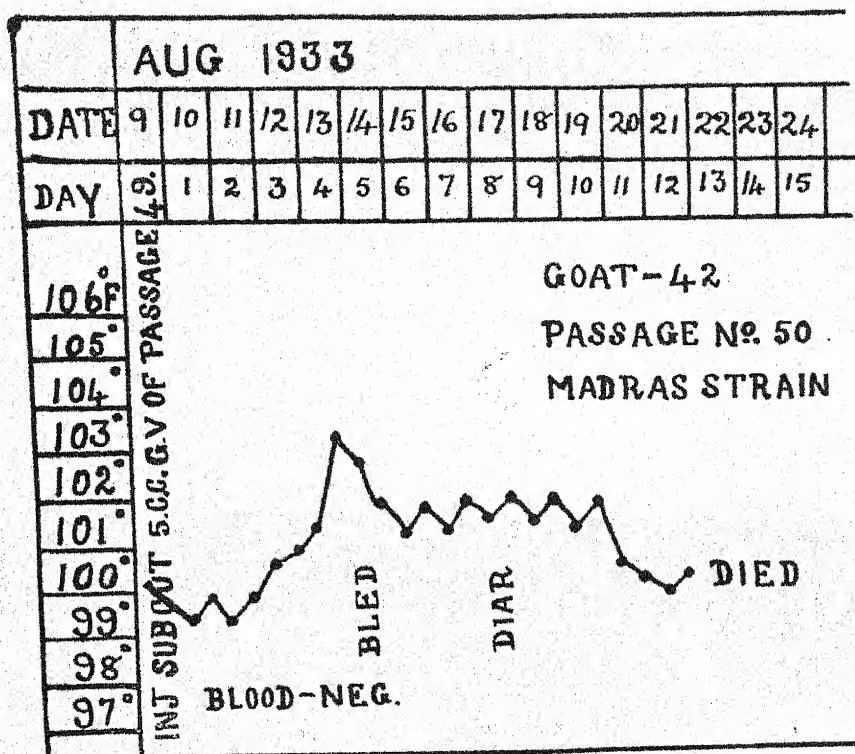
Chart XX.

Chart XXI.
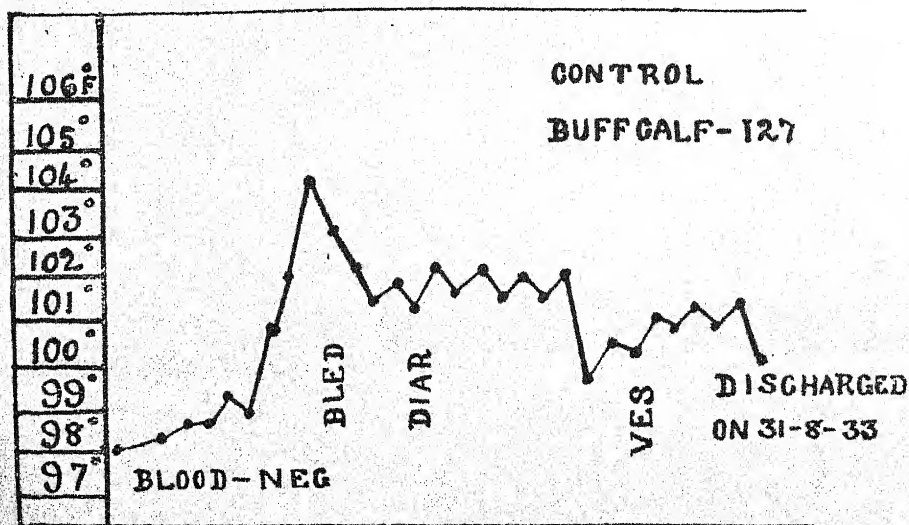
Chart XXII.

Chart XXIII.

Chart XXIV.

OCT 33													
DATE	17	18	19	20	21	22	23	24	25	26	27	28	29
DAY	59.	1	2	3	4	5	6	7	8	9	10	11	12

GOAT - 71.

PASSAGE Nº 60.

MADRAS STRAIN

INJ SUBCUT 5.C.C.G.V. OF PASSAGE

106°F 105° 104° 103° 102° 101° 100° 99° 98° 97°

BLED   DIAR   DIED

BLOOD— NEG.

Chart XXV.

106°F 105° 104° 103° 102° 101° 100° 99° 98° 97°

BLED

CONTROL

BUFF CALF— 184.

DIAR   VES   DISCHARGED

ON 6-11-33.

BLOOD— NEG
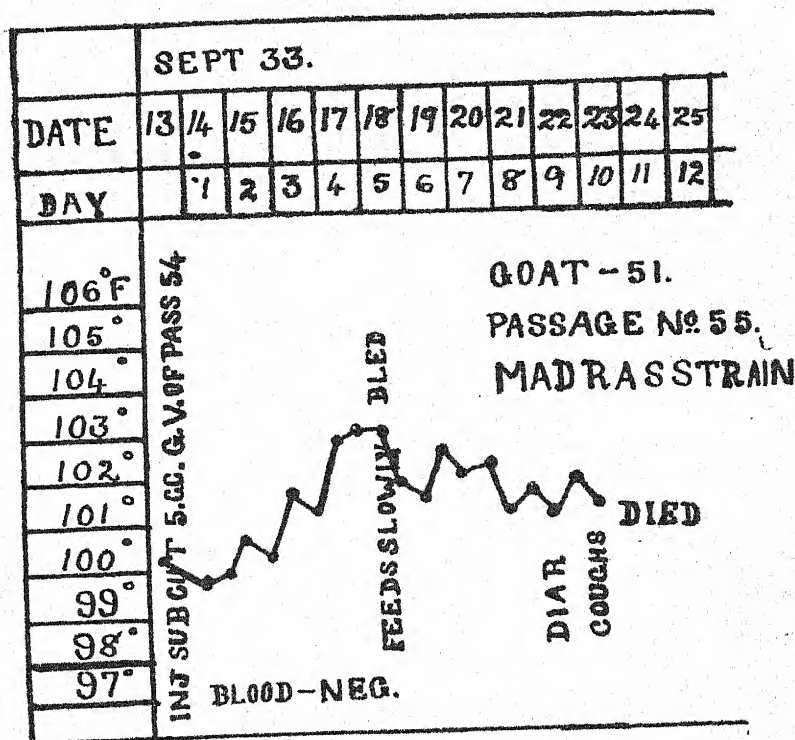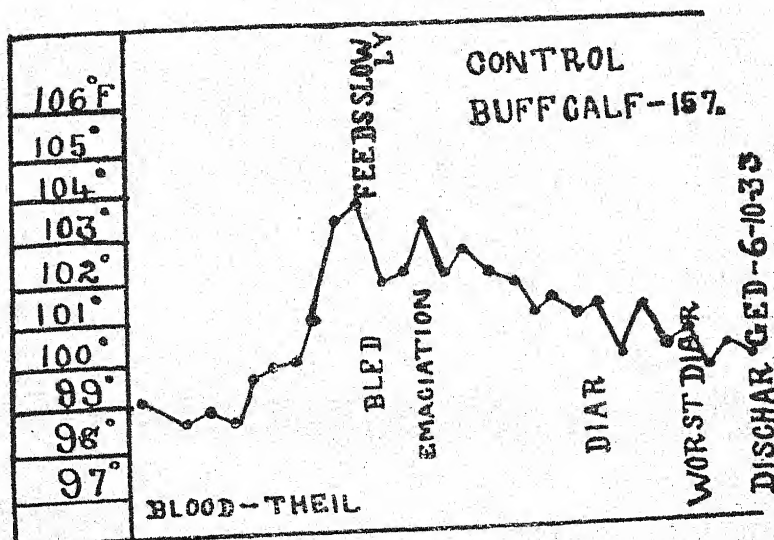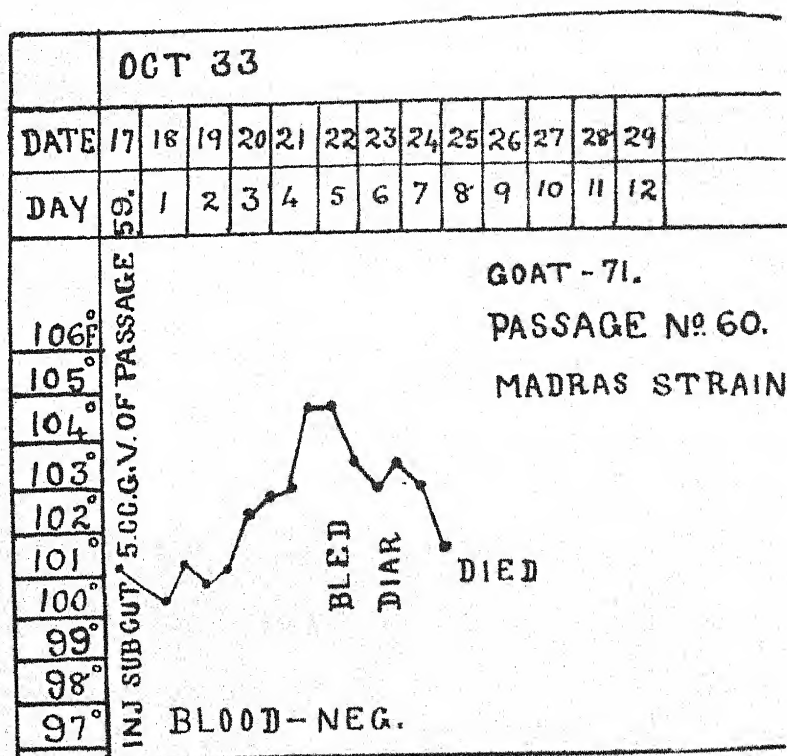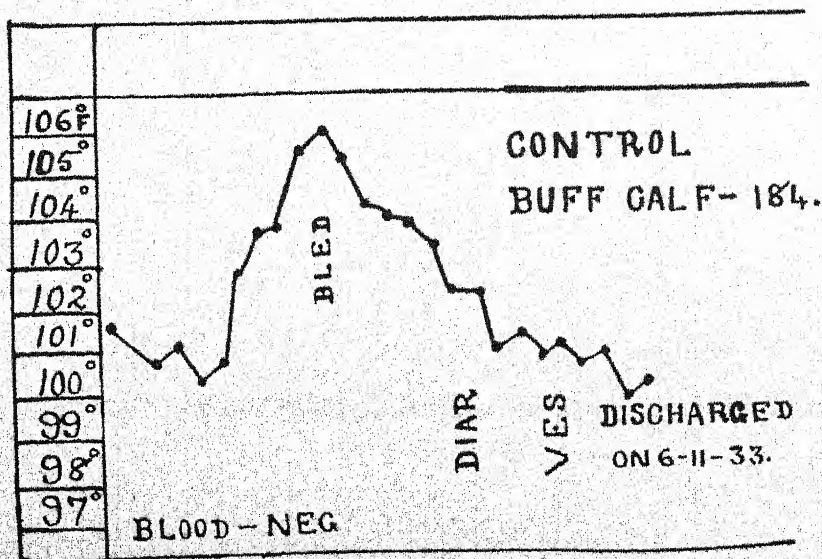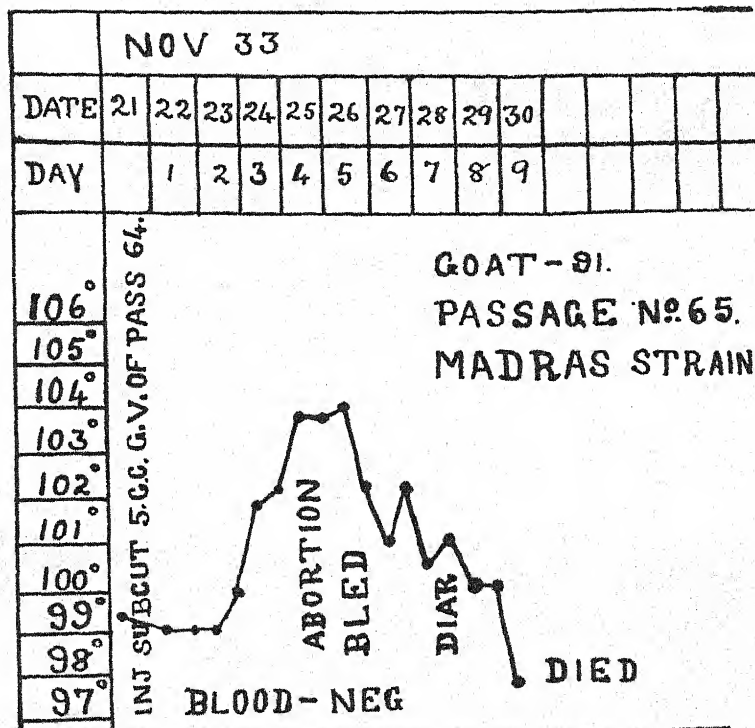
Chart XXVI.

Chart XXVII.

Chart XXVIII.

DEC 33.																
DATE	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5
DAY	69	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

GOAT - III.

PASSAGE № 70

MADRAS STRAIN

INJ SUB CUT 5.CC. G.V. OF PASSAGE

BLED

ABORTION

DIAR

BLOOD — NEG   DIED

106°F 105° 104° 103° 102° 101° 100° 99° 98° 97°

Chart XXIX.

CONTROL

BUFF CALF - 247.

BLED

VES   DIAR

BLOOD — THEIL.   DIED, 18-12-33

106°F 105° 104° 103° 102° 101° 100° 99° 98° 97°
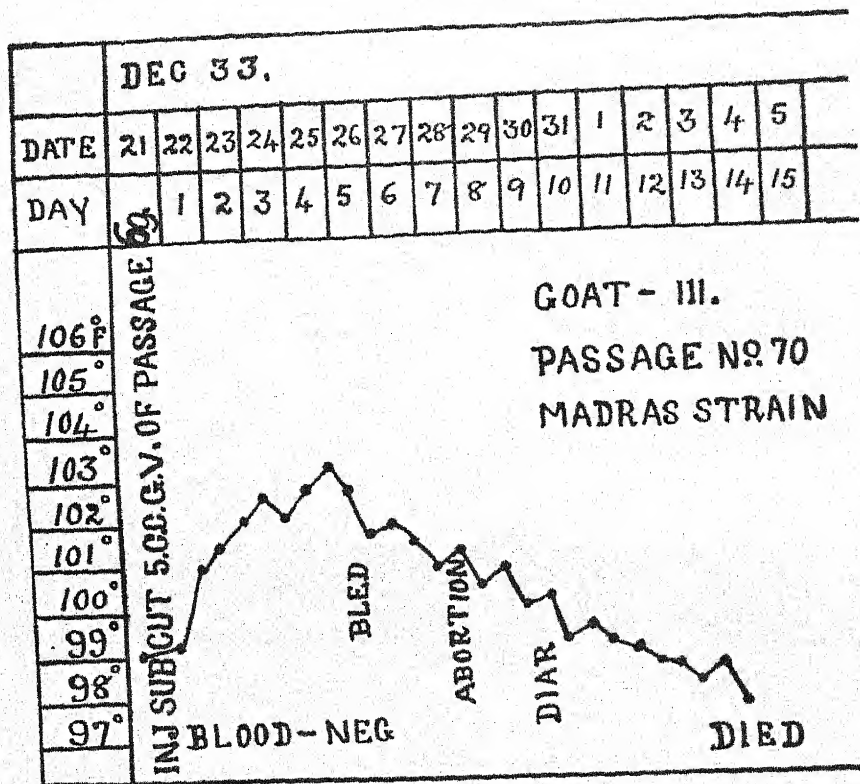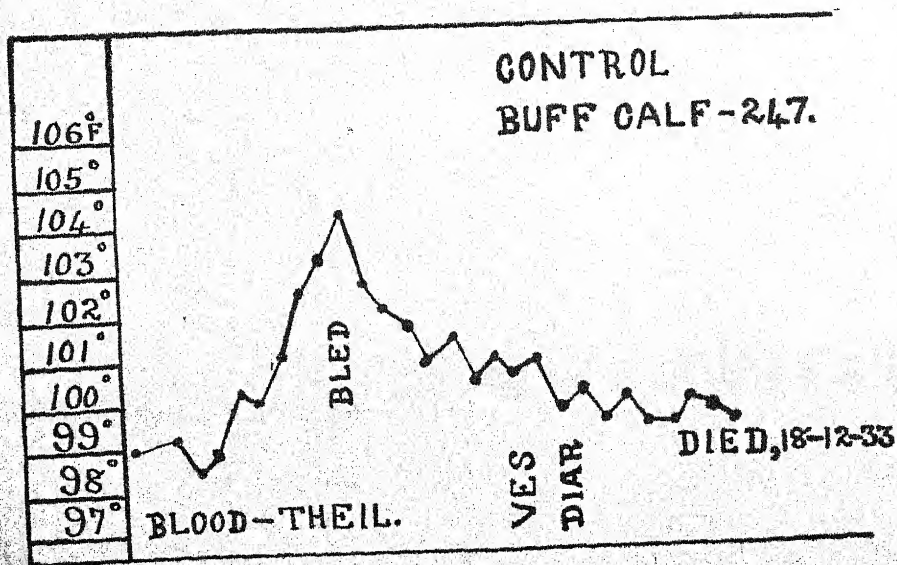
Chart XXX.

Chart XXXI.

Chart XXXII.

FEB – MAR 1934																
DATE	28	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
DAY		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

GOAT–156.
PASSAGE No. 80.
MADRAS STRAIN.

INJ SUB /CUT 5.CC. G.V. PASS:79

106°F
105°
104°
103°
102°
101°
100°
99°
98°
97°

BLED

DIAR

COUGH

DIED.

BLOOD – NEG

Chart XXXIII.

CONTROL BUFF CALF
321.

106°F
105°
104°
103°
102°
101°
100°
99°
98°
97°

BLED

DIAR

DISCHARGED
ON 18-3-34.

BLOOD – NEG

Chart XXXIV.

Chart XXXV.

Chart XXXVI.

MAY 34																
DATE	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
DAY		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

GOAT–19, PASSAGE Nº 90

MADRAS STRAIN

INJ SUBCUT 5CC. G.V. OF PASS–89

106°F 105° 104° 103° 102° 101° 100° 99° 98° 97°

BLOOD DRAWN

DIAR

DIED

BLOOD–NEG.

Chart XXXVII.

CONTROL–BUFF CALF 36

106°F 105° 104° 103° 102° 101° 100° 99° 98° 97°

BLED

DIAR

DISCHARGED
ON 23-5-34

BLOOD–THEIL
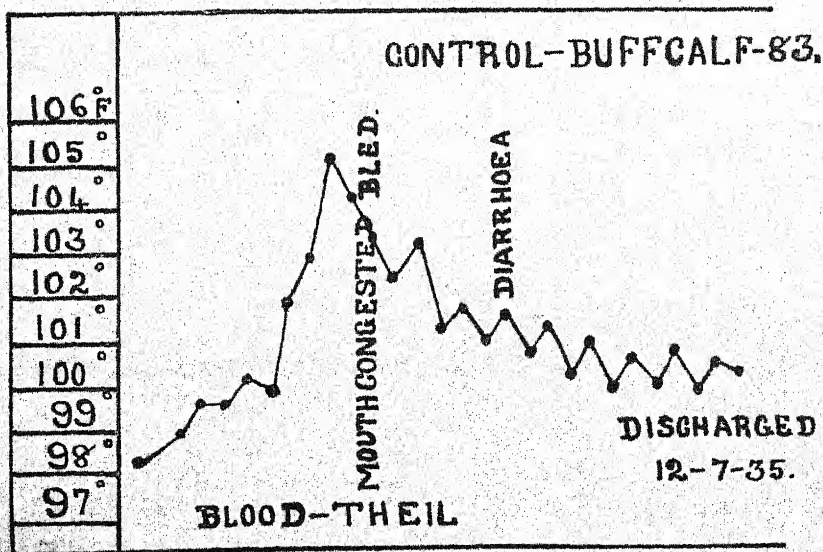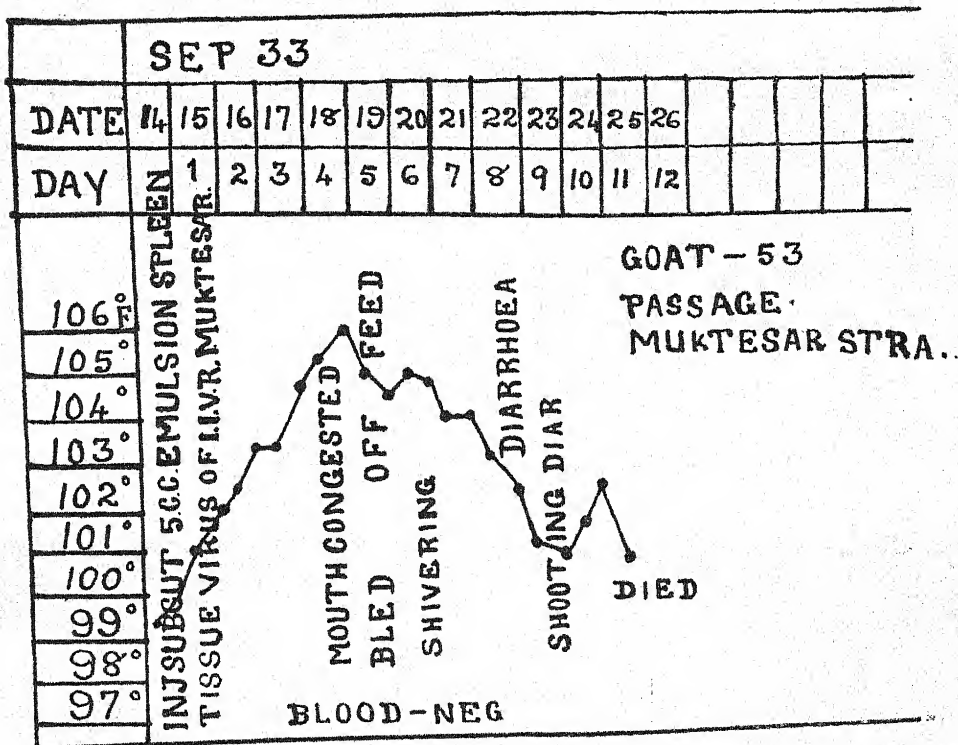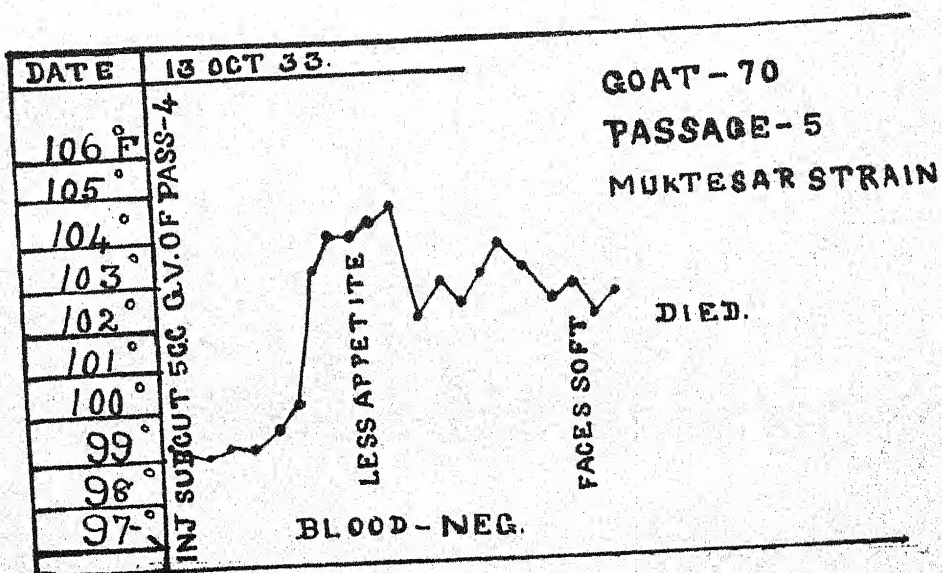
Chart XXXVIII.

Chart XXXIX.

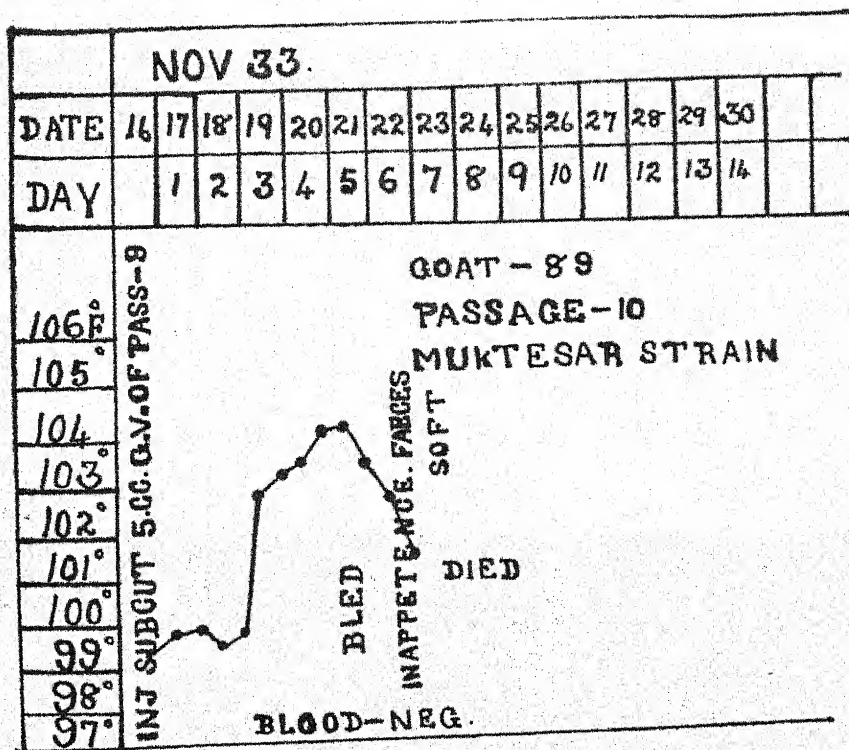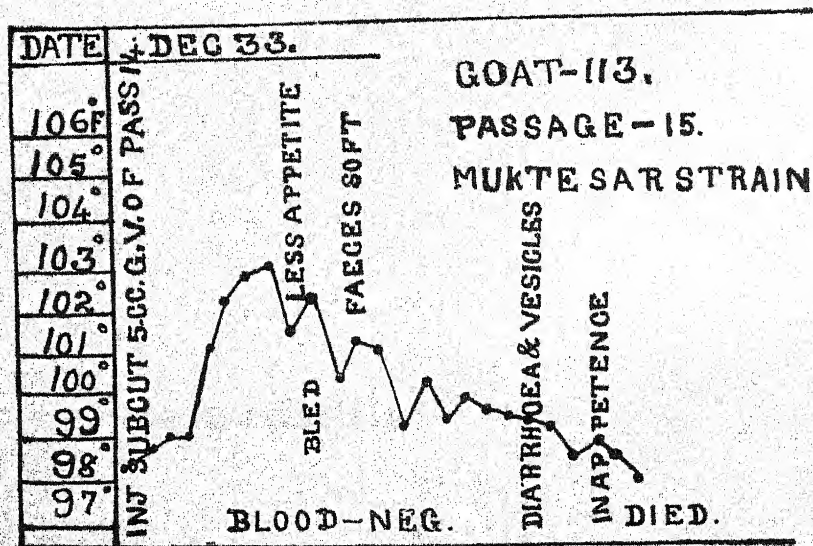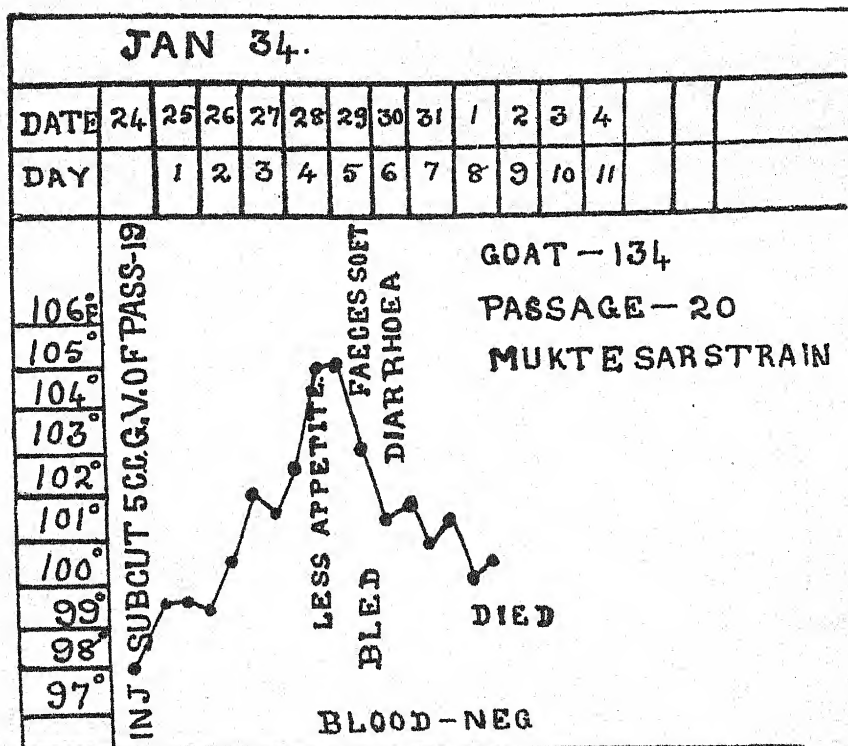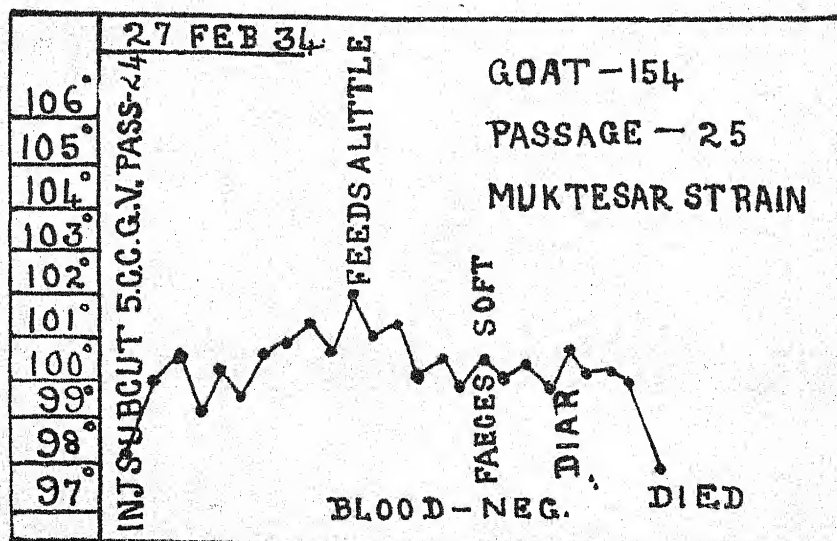

Chart XL.

Chart XLI.

Chart XLII.

Chart XLIII.

Chart XLIV.

Chart XLV.

Chart XLVI.

GOAT – 130.

PASSAGE – 115

MADRAS STRAIN

Chart XLVII.

CONTROL – BUFF CALF – 207.

Chart XLVIII.

NOV – DEC 34

DATE	30	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
DAY		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

INJ SUBCUT 5CC. C.V. OF PASS–119

SHIVERING.

BLED

DIARRHOEA

SHOOTING DIAR

DIED

BLOOD–NEG.

GOAT –151.
PASSAGE –120.
MADRAS STRAIN

Chart XLIX.

CONTROL BUFF CALF
241.

NASAL CATARRH

PURULENT DISCHARGE

INAPPETENCE

TESTED WITH BULL
VIRUS

DISCH – 23-12-34

BLOOD–THEIL

Chart L.

JAN 35.

DATE	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
DAY		1	2	3	4	5	6	7	8	9	10	11	12	13	14	

INJ SUBCUT 5.C.C., V. OF PASS-124.

CORYZA — SHIVERING — LESS APPETITE — BLED

DIED.

GOAT — 171.

PASSAGE — 125

MADRAS STRAIN

BLOOD — NEG.

Chart LI.

CONTROL — BUFF CALF — 269.

CORYZA — PURULENT DISCHAR — FAECES SOFT.

DISCHARGED

17-1-35.

BLOOD — THEIL.

Chart LII.

Chart LIII.

Chart content — FEB - 1935.

DATE	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
DAY		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

INJ SUBCUT 5.cc G.V. OF PASS-129.

GOAT - 200.
PASSAGE - 130.
MADRAS STRAIN

CORYZA
FEEDS SLOWLY
BLED
FAECES SOFT
DIED
BLOOD - NEG.

Chart LIV.

CONTROL - BUFFCALF - 295.

DISCHARGE FROM NOSE & EYES DULL

BLOOD - THEIL.

DISCHARGED 21-2-35.
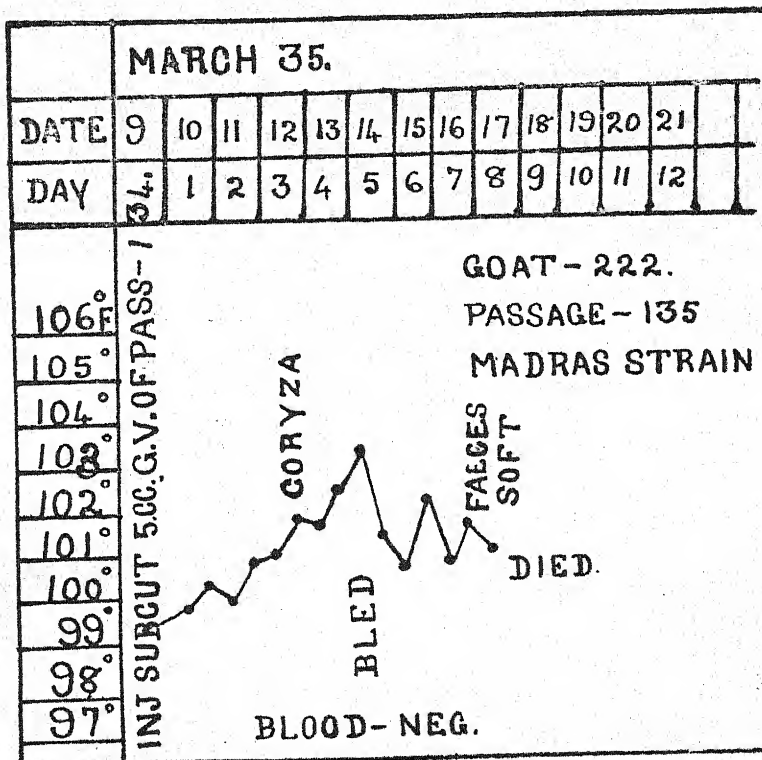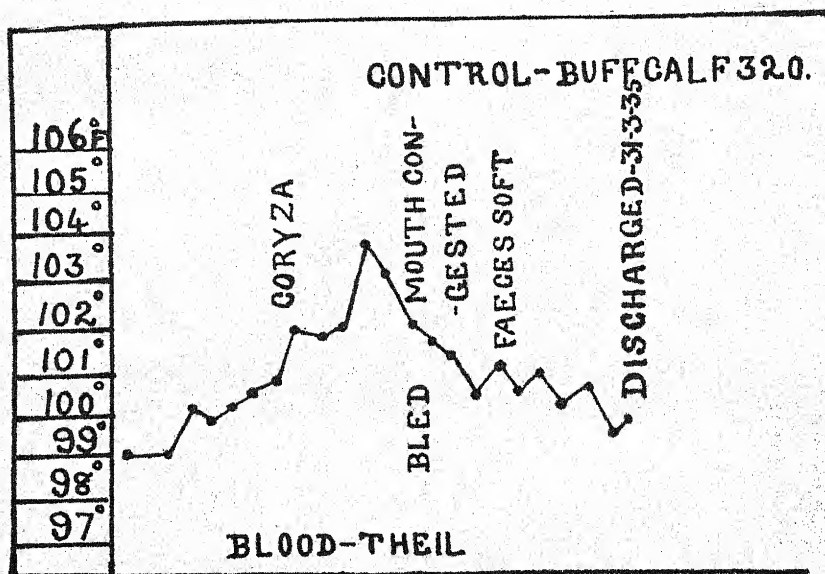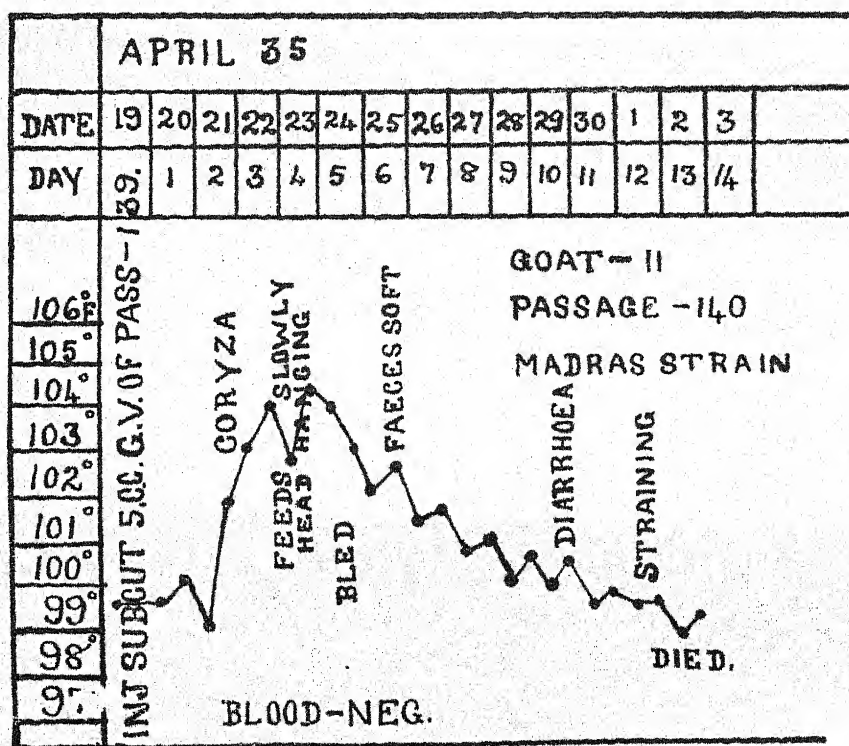
Chart LV.

Chart LVI.
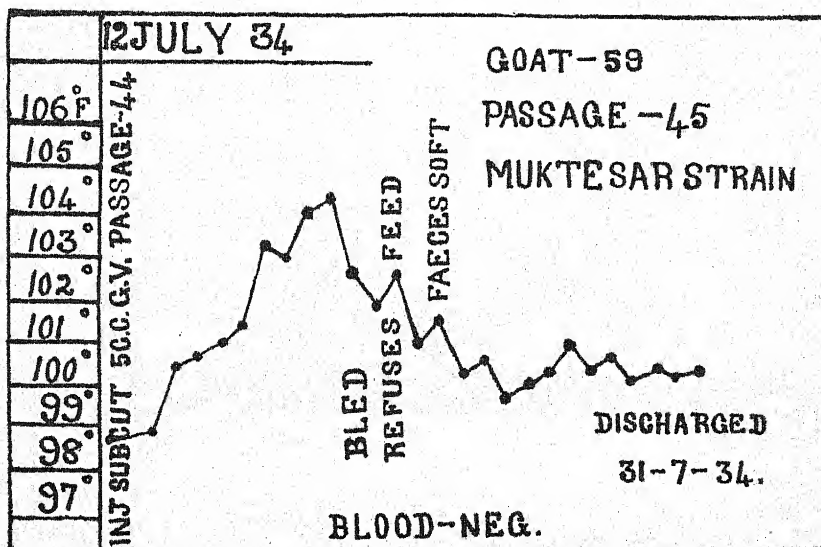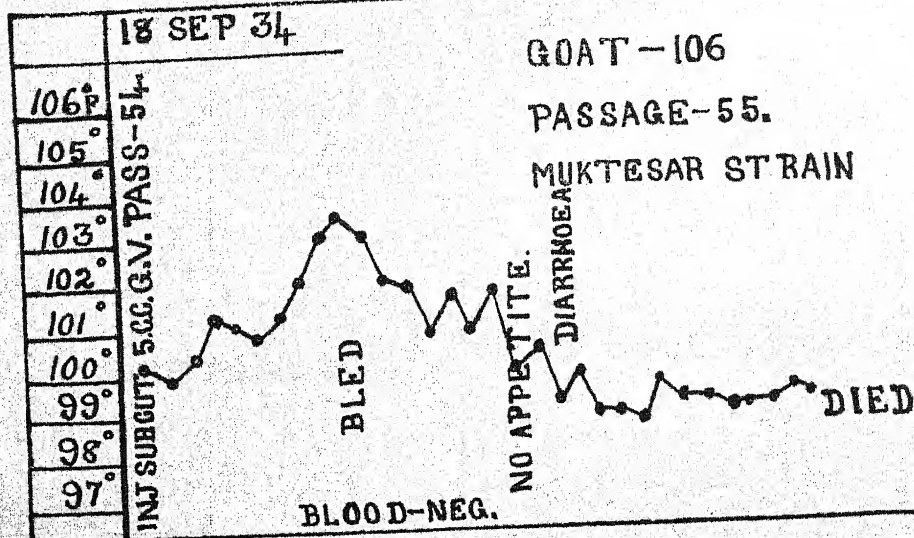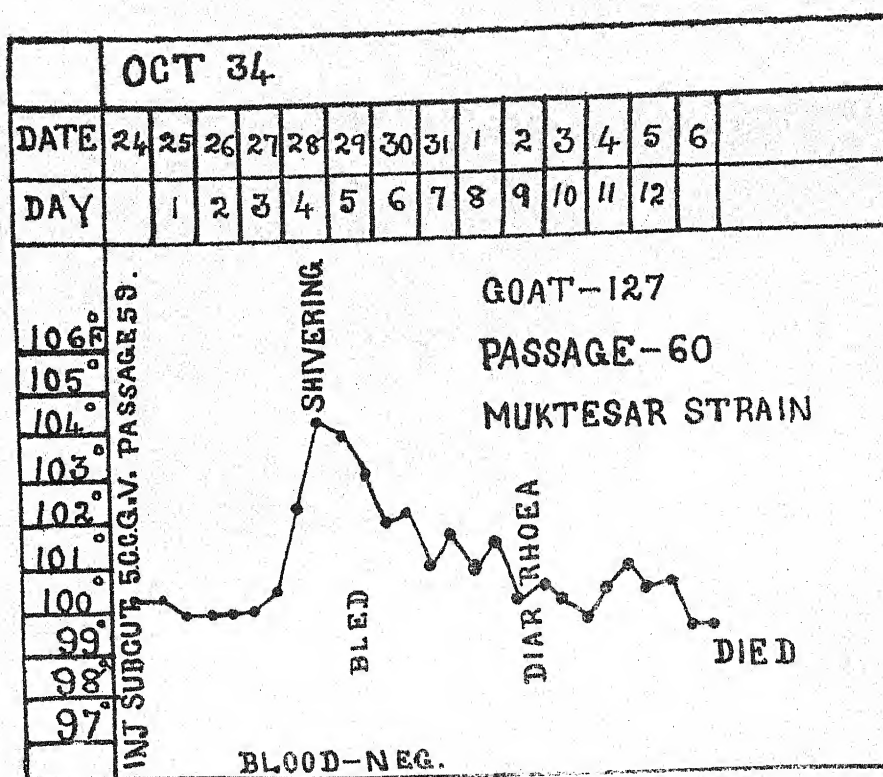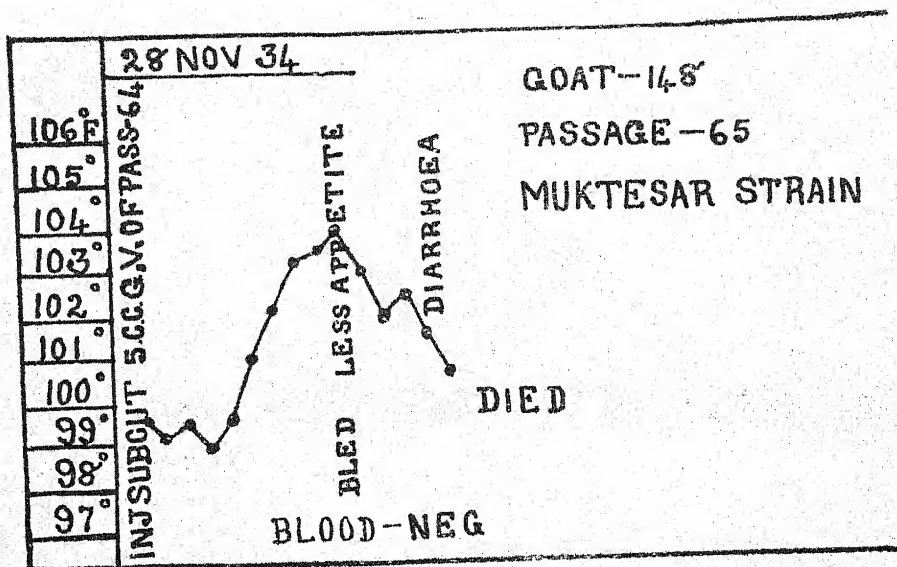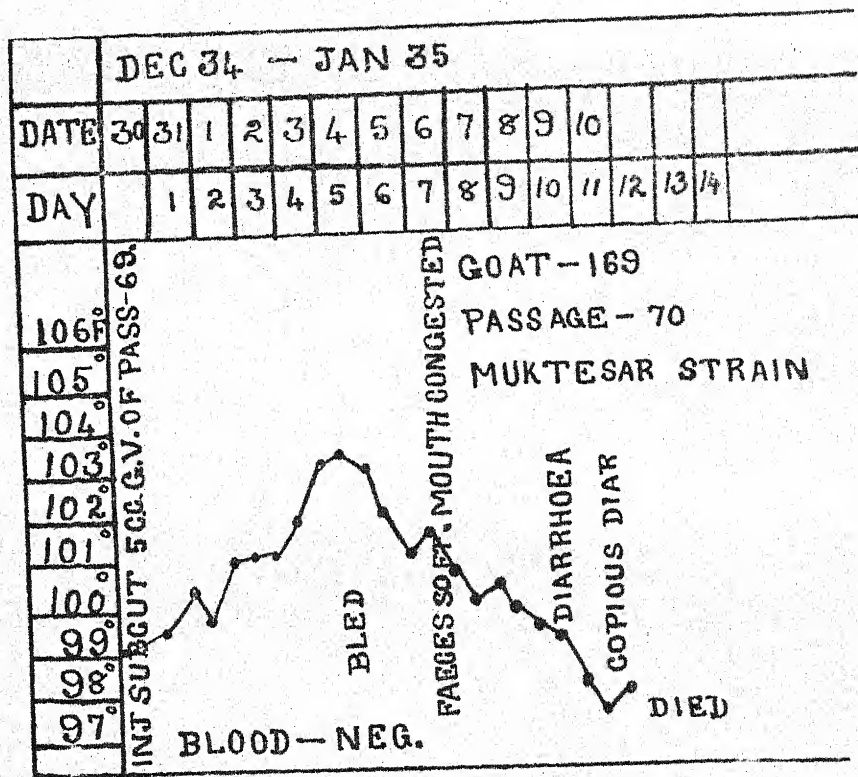
Chart LVII.
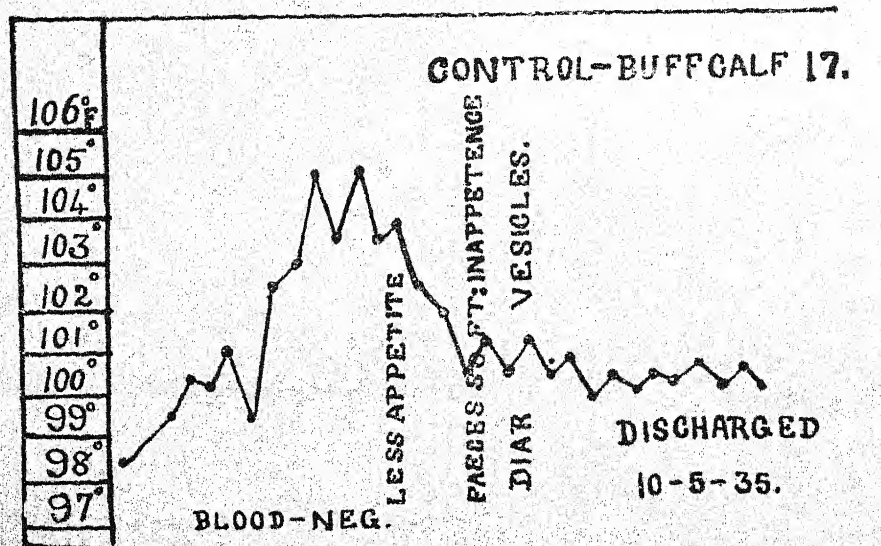
Chart LVIII.

Chart LIX.

Chart LX.

JUNE – JULY 35.														
DATE	27	28	29	30	1	2	3	4	5	6	7	8	9	10	11
DAY		1	2	3	4	5	6	7	8	9	10	11	12	13	14

INJ SUBCUT 5 CC. G.V. OF PASSAGE 149

106°F 105° 104° 103° 102° 101° 100° 99° 98° 97°

LACHRYMATION

NASAL CATARRH

HEAD HANGING

LESS APPETITE

BLED

GOAT— 56.

PASSAGE –150.

MADRAS STRAIN

DIED

BLOOD–NEG.

Chart LXI.

CONTROL—BUFFCALF–83.

106°F 105° 104° 103° 102° 101° 100° 99° 98° 97°

MOUTH CONGESTED BLED.

DIARRHOEA

DISCHARGED
12–7–35.

BLOOD–THEIL
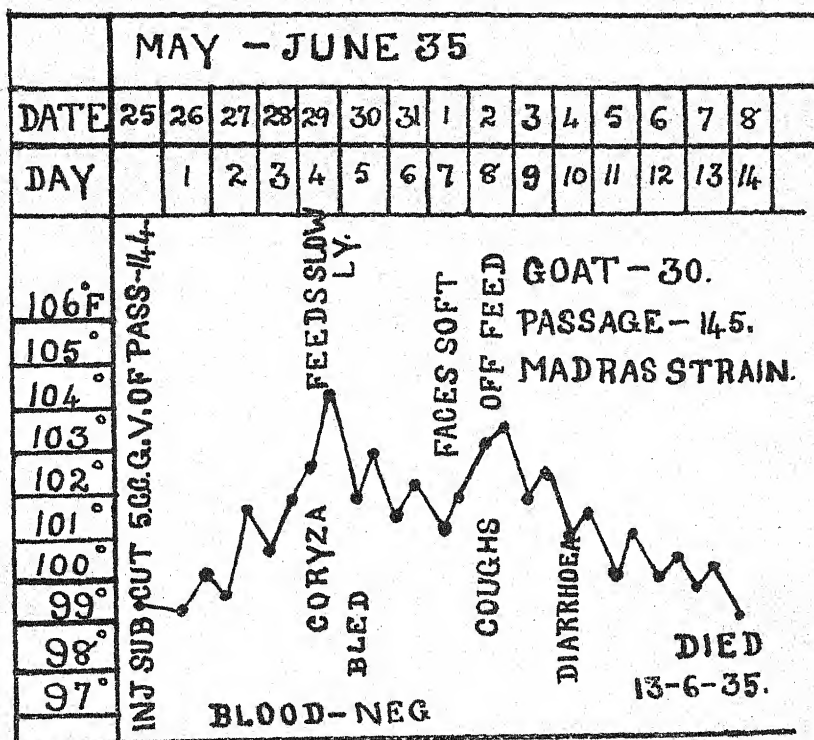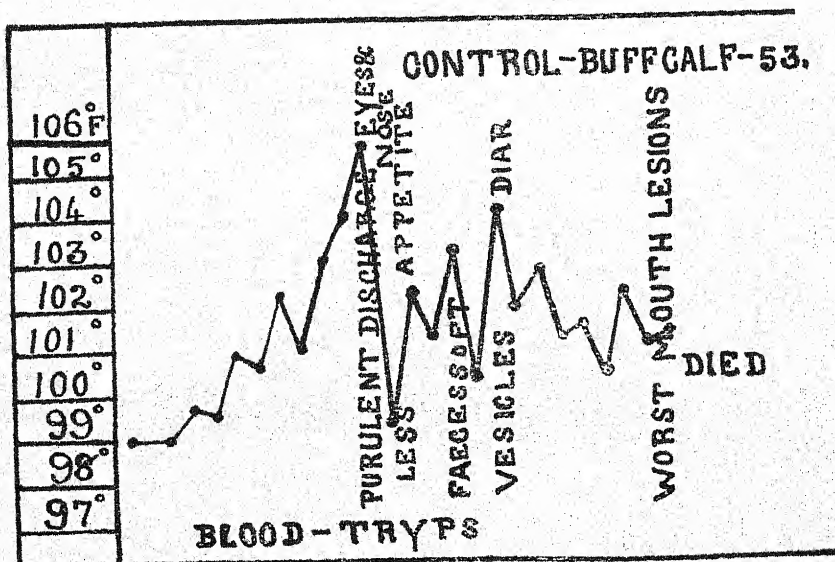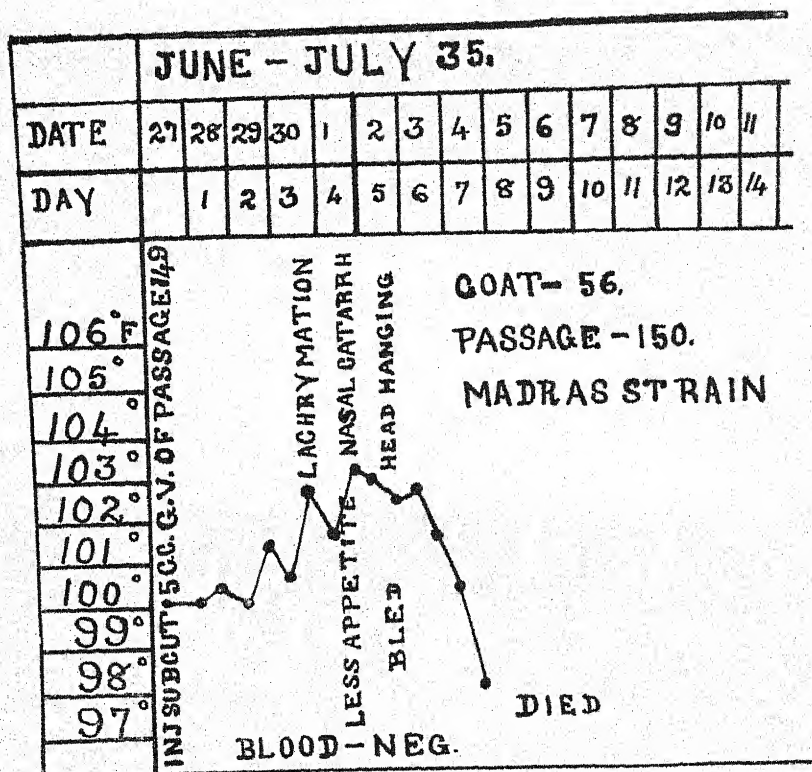
Chart LXII.

Chart LXIII.

Chart LXIV.

NOV 33.

DATE	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30		
DAY		1	2	3	4	5	6	7	8	9	10	11	12	13	14		

GOAT — 89
PASSAGE — 10
MUKTESAR STRAIN

INJ SUBCUT 5.CC. G.V. OF PASS—9

FAECES SOFT

BLED

INAPPETENCE.

DIED

BLOOD—NEG.

Chart LXV.

DATE	DEC 33.

GOAT — 113.
PASSAGE — 15.
MUKTESAR STRAIN

INJ SUBCUT 5.CC. G.V. OF PASS 14.

LESS APPETITE

FAECES SOFT

BLED

DIARRHOEA & VESICLES

INAPPETENCE

BLOOD—NEG.

DIED.

Chart LXVI.

JAN 34.

DATE	24	25	26	27	28	29	30	31	1	2	3	4			
DAY		1	2	3	4	5	6	7	8	9	10	11			

GOAT — 134
PASSAGE — 20
MUKTESAR STRAIN

INJ. SUBCUT 5 C.C.G.V. OF PASS-19

LESS APPETITE

FAECES SOFT
DIARRHOEA

BLED

DIED

BLOOD — NEG

Chart LXVII.

27 FEB 34.

GOAT — 154
PASSAGE — 25
MUKTESAR STRAIN

INJ. SUBCUT 5 C.C.G.V. PASS-24

FEEDS A LITTLE

FAECES SOFT

DIAR.

BLOOD — NEG.

DIED

Chart LXVIII.

MARCH - APRIL 34

DATE	30	31	1	2	3	4	5	6	7	8	9	10		
DAY			1	2	3	4	5	6	7	8	9	10		

GOAT — 174

PASSAGE — 30

MUKTESAR STRAIN

INJ SUBCUT 5.CC G.V. PASS — 29.

FEEDS SLOWLY

FAECES SOFT

FAECES VERY SOFT

BLED

DIED

BLOOD — NEG

Chart LXIX.

3 MAY 34

GOAT — 17

PASSAGE — 35

MUKTESAR STRAIN

INJ SUBCUT 5.CC G.V. PASS - 34

LESS APPETITE

FAECES SOFT

VERY SOFT

BLED

FAECES

DIED

BLOOD — NEG

Chart LXX.

Chart LXXI.

Chart LXXII.

Chart LXXIII.

Chart LXXIV.

Chart LXXV.

Chart LXXVI.

Chart LXXVII.

Chart LXXVIII.

Chart LXXIX.

Chart LXXX.

MAY 35.

| DATE | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | | | | |
| DAY | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |

GOAT–27
PASSAGE–90.
MUKTESAR STRAIN

INJ SUBCUT 5.C.C.G.V.OF PASS-89.

LESS APPETITE

FAECES SOFT

DIED

BLED

BLOOD–NEG.

Chart LXXXI.

25 JUNE

GOAT–53.
PASSAGE–95
MUKTESAR STRAIN

INJ SUBCUT 5.C.C.G.V. PASS-94

OFF FEED

FAECES SOFT

BLED

DIED

BLOOD–NEG.
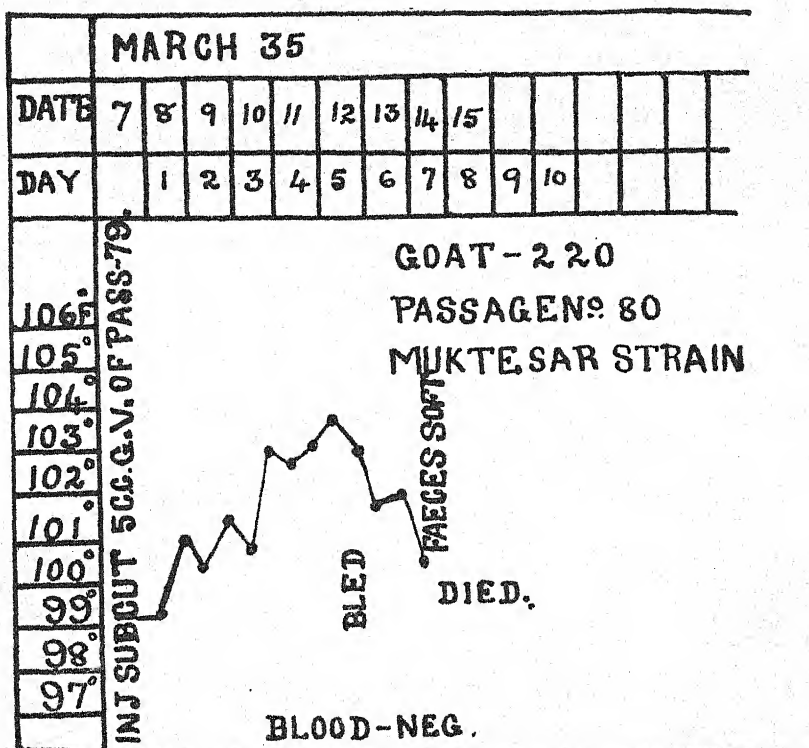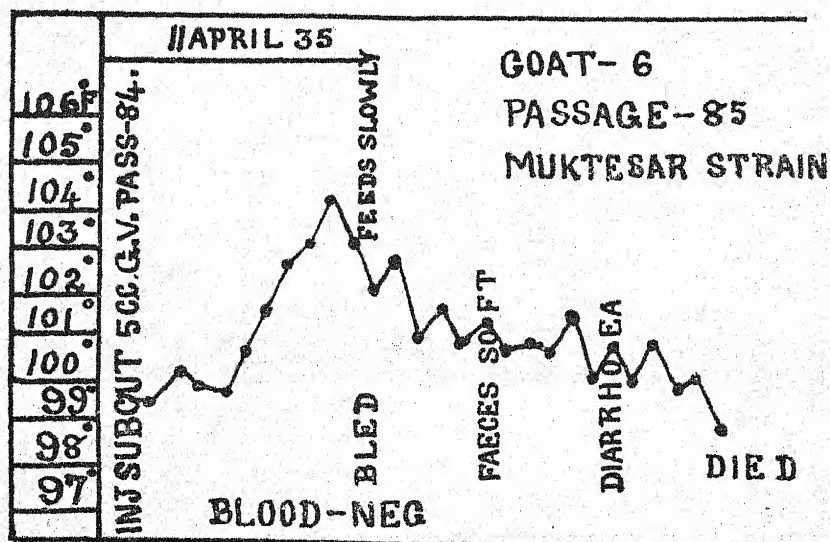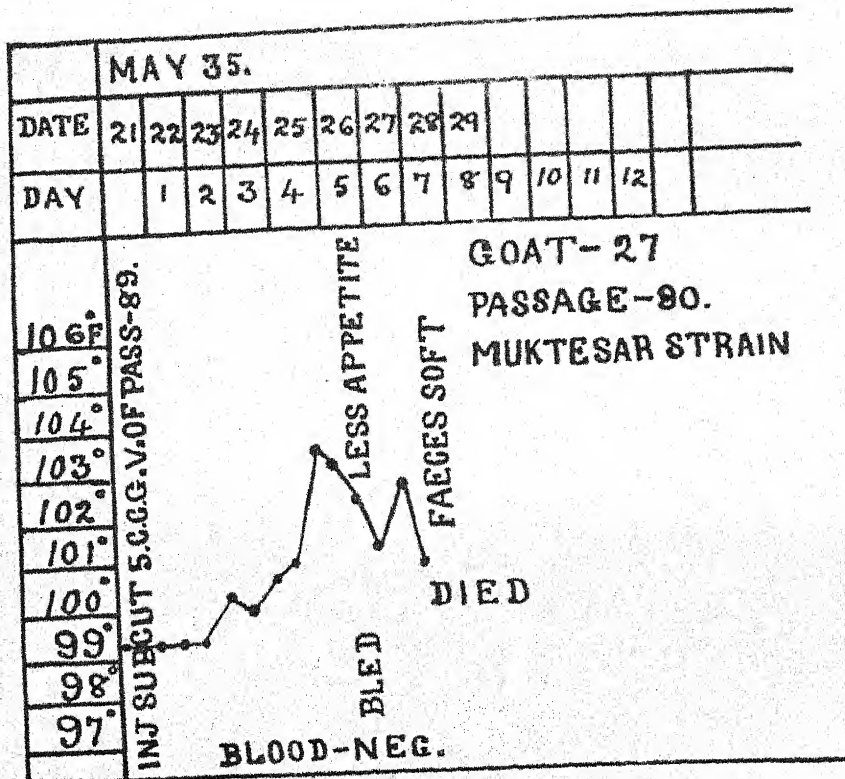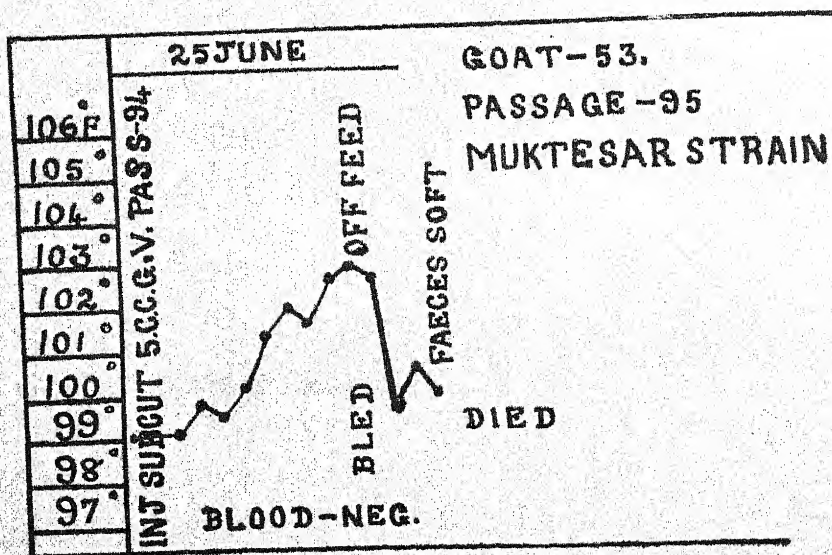
Chart LXXXII.

STUDIES ON THE DETERMINATION OF DIGESTIBILITY COEFFICIENTS :

II. THE ESTIMATION AND COMPUTATION OF DIGESTIBILITY COEFFICIENTS FROM INDIVIDUAL TESTS AND THEIR ORDER OF PRECISION

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INTRODUCTION

In a recent paper by Carbery, Chatterjee and Hye [1934], a method of experimentation and computation has been described, which is likely to ensure a greater degree of accuracy in arriving at the digestibility values of individual feeds and their components in a mixed ration. The chief point in favour of this method is that it enables direct estimation of the digestibilities without having recourse either to the use of assumed values (from standard books) for one or other items of the mixed feed, or to the alternative of conducting the trial with a single feed in the first instance, followed by the combined feed.

The method developed by the above authors is the result of an elaborate test involving eighteen individual estimations conducted in a cyclic order on six animals in a restricted randomised distribution. It need hardly be stated that the larger the number of tests and animals, the greater is the accuracy and representative character of the mean values. At the same time it cannot be overlooked that an increasing number of tests implies increasing outfits in staff, material and expenses. Thus, in many cases, it is not possible to have a large number of tests owing to shortage of men, money and materials.

On such an occasion a shorter method can be the only possible way to meet such special requirements. The development of a method giving a reasonable order of accuracy in such a contingency will be of great practical value. Naturally since the initiation of original tests this aspect engaged the close attention of the authors, and in the present paper a partial solution of this complicated problem

has been attempted by directing attention to two main lines of approach, *viz.*, (1) the method of computation and (2) the minimum number of tests necessary for a certain specified standard of accuracy.

METHOD OF COMPUTATION

With respect to the method of computation a mathematical formula can be satisfactorily worked out, in fact, has been done. It is based, of course, on the assumption that the behaviour of the animals is uniform. This assumption is implicit in all digestion tests and has been made by all investigators. If the animals were perfectly uniform, the data obtained from a test performed on one single animal would be quite sufficient to work on this formula.

In such a case the equation can be set up on the following basis. If R and C represent the amount of roughage and concentrate actually consumed and if D be the amount digested from the combined feed, then in a course of two digestion trials we get the following simultaneous equations :—

$$R_1x + C_1y = D_1 \quad . \quad . \quad . \quad (1)$$
$$R_2x + C_2y = D_2 \quad . \quad . \quad . \quad (2)$$

where x and y are the digestibility values (unitary basis) of R and C, and are assumed to be constant.

By solving these two equations we get :—

$$x = \frac{D_1C_2 - D_2C_1}{R_1C_2 - R_2C_1} \quad . \quad . \quad (3)$$

$$y = \frac{D_2R_1 - D_1R_2}{R_1C_2 - R_2C_1} \quad . \quad . \quad (4)$$

Similarly, if there are three feeds, say R, C and F and if x, y and z be their digestibility coefficients (unitary basis) then in the course of three digestion trials, we obtain the following three equations :—

$$R_1x + C_1y + F_1z = D_1 \quad . \quad (5)$$
$$R_2x + C_2y + F_2z = D_2 \quad . \quad (6)$$
$$R_3x + C_3y + F_3z = D_3 \quad . \quad (7)$$

By solving these we obtain the following values which for convenience are shown in terms of determinants :—

$$x = \begin{vmatrix} D_1 C_1 F_1 \\ D_2 C_2 F_2 \\ D_3 C_3 F_3 \end{vmatrix} \div \begin{vmatrix} R_1 C_1 F_1 \\ R_2 C_2 F_2 \\ R_3 C_3 F_3 \end{vmatrix} \quad . \quad . \quad (8)$$

$$y = \begin{vmatrix} R_1 D_1 F_1 \\ R_2 D_2 F_2 \\ R_3 D_3 F_3 \end{vmatrix} \div \begin{vmatrix} R_1 C_1 F_1 \\ R_2 C_2 F_2 \\ R_3 C_3 F_3 \end{vmatrix} \quad , \quad . \quad (9)$$

$$z = \begin{vmatrix} R_1 C_1 D_1 \\ R_2 C_2 D_2 \\ R_3 C_3 D_3 \end{vmatrix} \div \begin{vmatrix} R_1 C_1 F_1 \\ R_2 C_2 F_2 \\ R_3 C_3 F_3 \end{vmatrix} \quad . \quad . \quad (10)$$

In this way, we can obtain the individual digestibility of two, three or more feeds by conducting two, three or more trials depending on the number of feeds used. Theoretically, such a procedure would be free from any blemishes, if at the same time it satisfies the primary condition implicit in such an experiment, *viz.*, that the animal used behaved with mathematical uniformity. This, however, is next to impossible in actual practice. We have, therefore, to ascertain, what should be the minimum number of animals compatible with a reliable estimate of digestibility coefficient.

From the statistical standpoint, it is necessary to estimate the digestibility coefficient independently from a number of animals and then test the agreement between the results obtained. The closer the agreement, the greater the precision of individual estimates, hence the smaller the number of animals (*i.e.*, replications) required for the test. But we must theoretically have, at least a duplication, if not more to obtain an estimate of the precision of a single observation. This principle of replication must be kept in view to obtain an objective estimate of the precision in all possible cases and it is exactly for this purpose that field experiments in agriculture are always replicated. There are, however, instances where the test has been confined to one single animal. In such cases, the calculation of digestibility on the basis of the above equation can be the only alternative, despite its serious inherent limitations.

It will, however, be obvious that being based on the results of a single animal (in other words single observation in the statistical sense), it will not be possible to determine the extent of reliability of the digestibility coefficients. If, therefore, one or other sets of observations deviate appreciably from the normal conditions, the estimates of digestibilities are bound to be correspondingly affected without there being any means to detect their extent. Similarly, if the sets happen to be normal ones, the results are expected to be as much satisfactory as is possible within the range of the experiment. Such uncertainties in the calculation of digestibilities should, however, be avoided, as far as possible, in scientific investigations.

We should, therefore, have some definite knowledge as to what should be the minimum number of replications necessary to attain a certain specified standard of statistical accuracy. This will naturally imply a large number of individual tests. We have a fairly large number of data from another experiment leading to the paper on the estimation of digestibility coefficients [1934] and an analysis of the same would serve as a proximate index of the extent of deviation of a single observation.

It will, therefore, be necessary at this stage to give a short outline of the experiment conducted with regard to the above paper. This experiment was based on graphical representation and multiple regression equations with respect to two feeds, *viz.*, *aman* paddy straw and linseed cake, the latter being in doses of 1 lb.,

2 lb. and 3 lb. combinations, distributed in cyclic orders on the same six animals $(D_1, D_2, D_3, D_6, D_7, D_8)$ thereby providing eighteen separate figures for the amounts consumed and digested—six under 1 lb., six under 2 lb., and six under 3 lb. cake. For details, the reader is referred to Part I of this paper published in this Journal, Vol. IV, P. 295—340. Suffice it to state here that for the computation of digestibilities, x and y, as set forth under the equations (3) and (4), we require only two sets of data from a course of two feedings. Since, however, each individual animal was in turn under 1 lb., 2 lb. and 3 lb. combinations of cake, it provides three sets of data enabling three sets of computations for each individual animal, viz., (1) in which the equation can be worked with the data of 1 lb. and 2 lb., (2) with the data for 1 lb. and 3 lb. and (3) with the data for 2 lb. and 3 lb. As an illustration, one of the individual animals, D_1 may be taken. It consumed and digested the following amounts of crude protein during the three courses of feeding trial :—

TABLE I

Crude protein

Combination of feeding	Consumed		D Digested
	R Straw (grm.)	C Cake (grm.)	
1	2	3	4
1 lb. group	151·16	122·51	119·59
2 lb. group	167·52	261·96	238·05
3 lb. group	154·85	394·03	348·46

By applying, from the above table, the values of 1 lb. and 2 lb. combinations on formulae (3) and (4), we get the digestibilities of straw and cake as follows :—

$$x = \frac{119\cdot59 \times 261\cdot96 - 238\cdot05 \times 122\cdot51}{151\cdot16 \times 261\cdot96 - 167\cdot52 \times 122\cdot51} = 0\cdot11346 \qquad (11)$$

$$y = \frac{238\cdot05 \times 151\cdot16 - 119\cdot59 \times 167\cdot52}{151\cdot16 \times 261\cdot26 - 167\cdot52 \times 122\cdot51} = 0\cdot83617 \qquad (12)$$

Again, by applying the values under 1 lb. and 3 lb. combinations on the same formulae, we get the digestibilities :—

$$x = \frac{119 \cdot 59 \times 394 \cdot 03 - 348 \cdot 46 \times 122 \cdot 51}{151 \cdot 16 \times 394 \cdot 03 - 154 \cdot 85 \times 122 \cdot 51} = 0 \cdot 10919 \qquad (13)$$

$$y = \frac{348 \cdot 46 \times 151 \cdot 16 - 119 \cdot 59 \times 154 \cdot 85}{151 \cdot 16 \times 394 \cdot 03 - 154 \cdot 85 \times 122 \cdot 51} = 0 \cdot 84144 \qquad (14)$$

Similarly, by applying the values under 2 lb. and 3 lb. combinations, we get the digestibilities :—

$$x = \frac{238 \cdot 05 \times 394 \cdot 03 - 348 \cdot 46 \times 261 \cdot 96}{167 \cdot 52 \times 394 \cdot 03 - 154 \cdot 85 \times 261 \cdot 96} = 0 \cdot 09890 \qquad (15)$$

$$y = \frac{348 \cdot 46 \times 167 \cdot 52 - 238 \cdot 05 \times 154 \cdot 85}{167 \cdot 52 \times 394 \cdot 03 - 154 \cdot 85 \times 261 \cdot 96} = 0 \cdot 84548 \qquad (16)$$
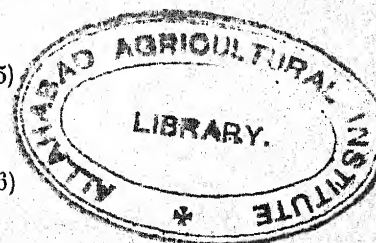
For a better presentation the above figures are set forth in the following table.

TABLE II

Combination of group	Digestibilities	
	Straw	Cake
1	2	3
1 lb. and 2 lb.	0·11346	0·83617
1 lb. and 3 lb.	0·10919	0·84144
2 lb. and 3 lb.	0·09890	0·84548

EXPERIMENTAL RESULTS

In this way we have been enabled to obtain eighteen (6×3) separate digestibility values for each of the food components (*viz.*, dry matter, organic matter, crude protein, ether extract, crude fibre and nitrogen-free extract) from the six animals experimented upon. These have been analysed statistically, and in the following table the mean values by each set of computations are set forth with

their corresponding standard deviations. To enable comparison the corresponding values by the graphical method and multiple regression equation as developed in the earlier paper (*loc. cit.*) are also given.

TABLE III

			Paddy straw			Linseed cake		
	Basis of calculation	No. of observation	Mean Digestibility	Standard deviation		Mean Digestibility	Standard deviation	
				Actual	In % on mean		Actual	In % on mean
1	2	3	4	5	6	7	8	9
Dry matter.	1 & 2 lb.	6	0·46180	0·0552	11·95	0·58735	0·3140	53·46
	1 & 3 lb.	6	0·45450	0·0387	8·52	0·66942	0·1292	19·30
	2 & 3 lb.	6	0·41960	0·0723	17·23	0·78935	0·2604	32·99
	Mean	18	0·44529	0·0599	13·45	0·68202	0·2606	38·21
	Graphical Method.	18	0·45070			0·70100		
	Multiple Regression Equation.	18	0·45560			0·65150		
	Older method.					0·79000 (H. & M.)		
	1 lb. cake	6	0·44278					
	2 lb. cake	6	0·42053					
	3 lb. cake	6	0·41834					
Organic matter.	1 & 2 lb.	6	0·51460	0·0601	11·68	0·62205	0·3223	51·81
	1 & 3 lb.	6	0·50845	0·0420	8·26	0·68980	0·1552	22·50
	2 & 3 lb.	6	0·47180	0·0899	19·06	0·80995	0·3095	38·21
	Mean	18	0·49827	0·0696	13·97	0·70728	0·2898	40·97
	Graphical Method.	18	0·50734			0·72160		
	Multiple Regression Equation.	18	0·51032			0·67069		
	Older method—							
	1 lb. cake	6	0·49623					
	2 lb. cake	6	0·47390					
	3 lb. cake	6	0·47055					
Crude protein.	1 & 2 lb.	6	0·12670	0·0984	77·66	0·81020	0·0436	5·38
	1 & 3 lb.	6	0·12875	0·0414	32·16	0·85160	0·0476	5·59
	2 & 3 lb.	6	0·02987	0·1594	533·65	0·90305	0·0790	8·75
	Mean	18	0·09510	0·1200	126·32	0·95499	0·0699	7·33
	Graphical Method.	18	0·08735			0·84754		
	Multiple Regression Equation.	18	0·09898			0·84113		
	Old method—					0·89000 (H. & M.)		
	1 lb. cake	6	0·5905					
	2 lb. cake	6	−1·2730					
	3 lb. cake	6	−0·9120					

TABLE III—contd.

	Basis of calculation	No. of observation	Paddy straw			Linseed cake.		
			Mean Digestibility	Standard deviation		Mean Digestibility	Standard deviation	
				Actual	In % on mean		Actual	In % on mean
1	2	3	4	5	6	7	8	9
Ether Extract.	1 & 2 lb.	6	0·42380	0·1622	38·27	0·96495	0·1307	13·54
	1 & 3 lb.	6	0·42933	0·1077	25·09	0·96070	0·0587	6·11
	2 & 3 lb.	6	0·38307	0·2561	66·86	0·98392	0·1212	12·32
	Mean	18	0·41206	0·1868	45·33	0·96985	0·1353	13·95
	G r a phical Method.	18	0·43164			0·96356		
	M u l t iple Regression Equation.	18	0·43790			0·95600		
	Old me-thod—					0·89000 (H. & M.)		
	1 lb. cake	6	0·48598					
	2 lb. cake	6	0·53243					
	3 lb. cake	6	0·59379					
Crude fibre.	1 & 2 lb.	6	0·62755	0·0554	8·83	0·04150	0·058	254·94
	1 & 3 lb.	6	0·61833	0·0495	8·01	0·11485	0·5596	487·24
	2 & 3 lb.	6	0·57620	0·1033	17·93	0·66140	0·296	195·95
	Mean	18	0·60736	0·0768	12·65	0·27258	0·056	387·41
	Graphical Method.	18	0·61650			0·27290		
	M u l tiple Regression Equation.	18	0·61894			0·07709		
	Old me-thod—					0·57000 (H. & M.)		
	1 lb. cake	6	0·60692					
	2 lb. cake	6	0·58331					
	3 lb. cake	6	0·58251					
Nitrogen free Ex-tract.	1 & 2 lb.	6	0·48125	0·0738	15·33	0·44235	0·6188	139·89
	1 & 3 lb.	6	0·46583	0·0363	7·79	0·63245	0·1466	23·18
	2 & 3 lb.	6	0·40493	0·1588	39·22	0·87090	0·6430	73·83
	Mean	18	0·45067	0·1084	24·05	0·64887	0·5407	83·37
	G r a phical Method.	18	0·46172			0·67298		
	M u l tiple Regression Equation.	18	0·46352			0·61345		
	Old me-thod—					0·78000		
	1 lb. cake	6	0·45295					
	2 lb. cake	6	0·42152					
	3 lb. cake	6	0·42695					

DISCUSSION

Before these figures are scrutinised, it is necessary to bear in mind the admittedly inexact nature of the chemical method of analysis used for their estimation. We have, at present, little knowledge regarding the fate of the food materials and their respective components during the course of their digestive processes in the alimentary canal of the herbivora. What is under existing convention assumed as digested is really the difference between the feed and what is obtained as undigested residue in the faeces. This does not take into account the secondary transformations taking place during digestion, nor such fractions or parts of feed, which although evacuated may still be capable of digestion. There is then the fact that faeces also contain excretory waste products from the body in addition to the undigested residues of the food, and as Armsby [1910] has remarked "the presence of so-called metabolic products in the faeces may give rise to serious errors in the determination of digestibility of some ingredients in the food, notably fat and protein."

In another place, Armsby [1903] has remarked that in "extreme cases, absurd results are sometimes obtained such as negative digestibility or a digestibility greater than 100 per cent". He [1910] has further expressed an apprehension of "the possibility of conversion of members of one group of nutrients into those of others." In fact, the recent investigations of Woodman and Stewart [1932] on the mechanism of cellulose digestion (by fermentation), show that in the case of oil cake the ether extract fraction seems to undergo such transformations as to assume an insoluble nature thereby showing an apparent increase in fibre. While this refers to bacterial fermentation, the present authors feel that the possibility of some changes of like nature in the animal system are not unlikely, thus giving an apparent increase of the fibre content of faeces and correspondingly lowering or giving a negative value of the fraction representing digestion. At any rate, the process is full of complexities and they find ample illustration in the figures set forth in Table III. With respect to paddy straw the standard deviations for the digestibilities of dry matter, organic matter, crude fibre and to some extent Nitrogen-free Extract are comparatively small, ranging between 8 to 19 %. The corresponding standard deviations of the same components under cake are however much higher, being $19 \cdot 3$ to $38 \cdot 2\%$ for dry matter and $22 \cdot 5$ to $40 \cdot 97\%$ for organic matter, whereas it has been unusually high in the case of crude fibre, the climax being over 487% in one set (Table III, column 9).

Again the standard deviations for crude protein and ether extract under cake have been quite low, ranging from $5 \cdot 38$ to $8 \cdot 75\%$ in the former and $6 \cdot 11$ to $13 \cdot 95\%$ in the latter, whereas the same for paddy straw have been very high particularly with respect to crude protein where in one set it has worked out at $533 \cdot 65\%$ (Table III, column 6).

The divergencies are naturally indicative of both high and low rates of digestion, the higher values sometimes working out above cent per cent and the lower ones receding into negative values.

If we compare the standard deviations in relation to the procedure of calculations by the three different groupings, viz., 1 lb. and 2 lb., 1 lb. and 3 lb. and 2 lb. and 3 lb., we note that in the midst of all the divergencies, the standard deviations with respect to the calculations under 1 lb. and 3 lb. grouping, have been uniformly low, except in one solitary instance of crude fibre under cake. It might be noted here that the difference in the amount of cake consumed is largest in the case of 1 and 3 lb. as compared to 1 and 2 lb. or 2 and 3 lb. This might partially account for the low error, as in the estimate of digested amount (the equation being on a linear basis) a given error in the slope representing digestibility will necessarily be reduced when such difference is greater.

It need hardly be stated here that the figures have been worked out from the data of an experiment where every attempt was made to obtain reasonable uniformity in the selection of the animals, regarding age, live weights, feeding and other treatments. Yet it is very difficult from the very nature of the animals to maintain a strict order of uniformity. In other words there is bound to be some difference due to variation amongst the animals. The standard deviations in columns 5 and 8 in Table III, thus represent the total variability arising out of (1) experimental errors and (2) variations amongst the animals. As already stated, the standard deviations of some particular components such as crude protein of paddy straw and crude fibre of linseed cake are enormously high. As the experimental order of procedure was identical in all, the experimental errors are also expected to be of an identical order. It is unlikely that in these cases alone the experimental errors were particularly high. Assuming, therefore, a more or less similar order of experimental error, it may be tentatively suggested that the digestibility coefficients with regard to these components are more susceptible to variation than those of others. But unless the extent of this susceptibility is brought within the range of measurable estimation, it is bound to affect the accuracy in proportion to the degree of such susceptibility. To that extent the values obtained will necessarily be affected. Yet, until we have some means to eliminate such variability, we have to proceed on an assumption that the behavior of the animals is uniform. This assumption is as much implicit in the already prevailing method of computation as it is in the one now suggested.

It is obvious that if there is a mathematical uniformity in the process of digestion, the application of the equation (formulae 3 and 4) is fully justifiable and the results obtained even from a single animal would be fully dependable. Since however, strict uniformity in animals is out of the question in actual practice, the only alternative, as has been stated before is to replicate the experiment adequately in order that the mean values so obtained are dependable.

N

MINIMUM NUMBER OF TESTS NECESSARY FOR RELIABLE VALUES

This naturally leads to the question as to what should be the minimum number of replications to warrant a certain specified standard of statistical accuracy.

For this purpose we can use the well known formulae :—

$$E^2 = \frac{\sigma^2}{n}, \text{ where}$$

σ = Population value of the percentage standard deviation of the observed sample (for which the sample value is substituted in this case),

E = intended percentage error on mean, and

n = required number of replications for attaining the specified standard of accuracy.

Now, having regard to the many practical difficulties in the way of extensive replications, it cannot, very often, be a practical proposition in digestion experiments to aim at an error of very low denomination.

A moderately low error, say, from 5 to 15% may be considered to be quite reasonable as meeting the ordinary requirement ; and with that idea in view, the values have been worked out under three sets of errors, *viz.,* 5%, 10% and 15%. These are now set forth in the following table :—

TABLE IV

Basis of calculation	Paddy straw				Linseed cake			
	Standard deviation in % on mean	Number of replications necessary for an accuracy of			Standard deviation in % on mean	Number of replications necessary for an accuracy of		
		5%	10%	15%		5%	10%	15%
Dry matter 1 and 2 lb.	11·950	6	4	...	53·460	115	29	13
1 and 3 lb.	8·515	3	...	...	19·300	15	4	2
2 and 3 lb.	17·231	12	...	...	32·989	44	11	5
Organic matter. 1 and 2 lb.	11·679	6	2	...	51·813	108	27	12
1 and 3 lb.	8·260	3	...	...	22·499	21	6	3
2 and 3 lb.	19·055	15	4	2	38·212	59	15	7
Crude protein. 1 and 2 lb.	77·063	241	61	27	5·381	2	...	...
1 and 3 lb.	32·155	42	11	5	5·589	2	...	...
2 and 3 lb.	533·645	...	...	...	8·748	4	...	...
Ether Extract. 1 and 2 lb.	38·273	59	15	7	13·545	8	2	...
1 and 3 lb.	25·086	26	7	3	6·110	2	...	...
2 and 3 lb.	66·855	170	45	20	12·318	7	2	...
Crude fibre 1 and 2 lb.	8·828	4	...	...	254·94	?	?	?
1 and 3 lb.	8·005	3	...	...	487·24	?	?	?
2 and 3 lb.	17·928	13	4	...	195·95	?	?	?
Nitrogen free extract. 1 and 2 lb.	15·335	10	3	...	139·887	?	?	?
1 and 3 lb.	7·793	3	...	...	23·179	20	6	3
2 and 3 lb.	39·217	62	16	7	73·832	...	...	...

A study of these figures will show that the comparatively low standard deviation under the calculation basis of 1 lb. and 3 lb. combinations (a reference to which has already been made) have been correspondingly reflected in a lower number of required replications. For a better comparison they are reproduced in the following table :—

TABLE V

(1 lb. and 3 lb. basis of calculation.)

	Paddy straw				Linseed cake			
	Standard deviation in % on mean	Number of replications necessary for an accuracy of			Standard deviation in % on mean	Number of replications necessary for an accuracy of		
		5%	10%	15%		5%	10%	15%
Dry matter	8·515	3	...	...	19·300	15	4	2
Organic matter	8·260	3	...	...	22·499	21	6	3
Crude protein	32·155	42	11	5	5·589	2	...	...
Ether Extract	25·086	26	7	3	6·110	2	...	...
Crude fibre	8·005	3	...	...	487·24	?	?	?
Nitrogen-free extract . . .	7·790	3	...	...	23·179	22	6	3

As already stated, these results have been worked out from a rather limited number of requisite types of data. Nevertheless, they serve as actual illustrations of the possible order of uniformity or otherwise and the trend and possibility of the reduction in errors by means of a reasonable number of replications. We note here that with respect to four only of six components of paddy straw, *viz.*, dry matter, organic matter, crude fibre and nitrogen-free extract, not more than three replications would be necessary to keep the error within 5%. But crude protein and ether extract appear to be liable to greater fluctuations, in consequence of which even an error of 10% seems to be beset with practical difficulties, as even then it would require as many as 11 replications (7 for ether extract and 11 for crude protein). In fact in these two cases it may ordinarily be a more feasible course to be satisfied with an error as high as 15%, for it is only then that the replications can be kept within the moderate limit of 3 to 5.

In the case of linseed cake, crude protein and ether extract are the only two components whose errors are concomitant with low replications. For the values with 1 and 3 lb. basis of calculation even a replication of 2 is enough to keep the error within 5%, but in respect of other components the position is different. As many as 4 to 6 replications would be necessary in the case of dry matter, organic matter and nitrogen-free extract to keep the error within 10%, whereas, even this is not sufficient with respect to crude fibre.

It should be borne in mind, as has been mentioned already that with respect to crude fibre of linseed cake, and crude protein and to some extent ether extract of paddy straw, there are other factors eluding experimental control.   Leaving these exceptional cases, it may be broadly stated that if we set a limit of, say, 10% error, the maximum replication should be about six. If it is 15 %, the replication should be 3 for all, except with respect to crude protein of paddy straw, where with three replications the error will work out at a figure exceeding 25%. One will often have to be satisfied with such a large error when other considerations *will require us to limit* the number of replications to 3.

It is necessary to state here that apart from the limited available data with which the validity of the formulae has been tested, these figures are from an experiment conducted on three different planes of nutrition.   It has been assumed as a first approximation that the digestibilities at these different planes were the same and constant. It is not known how far the preponderance of one food-stuff reacts on those of the other.   Possibly a number of tests on the same plane of nutrition might provide data for testing the validity of the formulae on one side and the extent of difference in digestibility under the different planes of nutrition. For the present such figures are not available but if circumstances permit they will be tried.   In the meantime, the results so far obtained are placed before other workers so that, if possible, they may also give a trial to the procedure.

The results dealt with are with reference to two feeds on the basis of formulae 3 and 4. It has not been possible to try the formulae 8, 9 and 10 with respect to three feeds as the requisite data were not yet available but theoretically their validity rests on identical principles.

SUMMARY AND CONCLUSIONS

1. In view of contingencies where the amount of food material may not be sufficient for an extensive test, the admitted necessity of shorter method has led to two lines of approach, *viz.*, (i) method of computation and (ii) the minimum number of tests necessary for a certain specified standard of statistical accuracy.

2. The method of computation has led to the working out of the formulae :—

$$x = \frac{D_1 C_2 - D_2 C_1}{R_1 C_2 - R_2 C_1}$$

$$y = \frac{D_2 R_1 - D_1 R_2}{R_1 C_2 - R_2 C_1}$$

where x and y are the digestibilities (unitary basis) of two feeds R and C and D, their total amount actually digested. The calculations are based on two digestion tests indicated by suffixes in the above formulae. Similarly, if there are three feeds the calculation can be made with the data from three trials as set forth in formulae 8, 9 and 10 (*vide* text).

3. The minimum number of tests necessary for a desired standard of accuracy has been derived from the formulae $E^2 = \dfrac{\sigma^2}{n}$ where

$\sigma = \%$ standard deviation found

$E = \%$ standard deviation aimed

$n =$ number of replications.

4. The limited number of requisite data did not permit of a more extensive trial of the formulae, and even here it has been confined to a test on two feeds as no data with three feeds were available. The results have, however, been highly encouraging and it is found that, with respect to paddy straw and cake (two feeds), a fair order of accuracy is possible with a replication of 3 to 5, except in the case of crude protein and ether extract under paddy straw and crude fibre under linseed cake.

5. The method of computation enables us to set a direct evaluation of individual digestibilities and thus obviates the necessity of conducting separate trials with single feeds followed by combined feeds.

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A SEVERE AND WIDESPREAD OUTBREAK OF RINDER-PEST AT THE GOVERNMENT CATTLE FARM, HISSAR, AND ITS CONTROL BY THE GOAT-VIRUS-ALONE METHOD

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INTRODUCTION

In the heart of Hissar district, with an area of some sixty-three square miles, is situated the Government Cattle Farm, Hissar, where for many years cattle of the finest Indian breed have been reared by the Punjab Government. The *Bir* or grass-lands of this farm are of very wide extent and cover an area of about 21,000 acres. The number of livestock on this farm amounts to several thousand heads.

The farm stock is distributed in different places, at a distance of several miles from one another. The distribution is given below :—

1. *Home Farm.* All the stock requiring special care and supervision, such as dairy cows, standard cows, special cows and weak and debilitated animals.

2. *Sally Farm.* The general herd of cows along with their bulls and young progeny.

3. *Thaska Farm.* The white herd of cows along with their bulls and young progeny.

4. *Kherwan Farm.* The heifers from their weaning age till their maturity which is 3 years.

5. *Chowni Farm.*—The male stock till they mature into bulls when they are issued to the districts for distribution at the age of $2\frac{1}{2}$ years.

6. *Bobran Farm.*—The grey herd is located here in the rainy season and throughout the summer months until the cold weather starts.

Fig. I shows the distribution of the livestock at different places, and the roads connecting them.

KHERWAN
FEMALE PRODUCE

THASKA
WHITE BREEDING HERD
SUMMER

SULLY
MAIN BREEDING HERD
WINTER

BOBRAN
MAIN BREEDING HERD
SUMMER.

HISSAR CITY

CHOWNI
MALE PRODUCE

HOME FARM
DAIRY & SELECTED STOCK
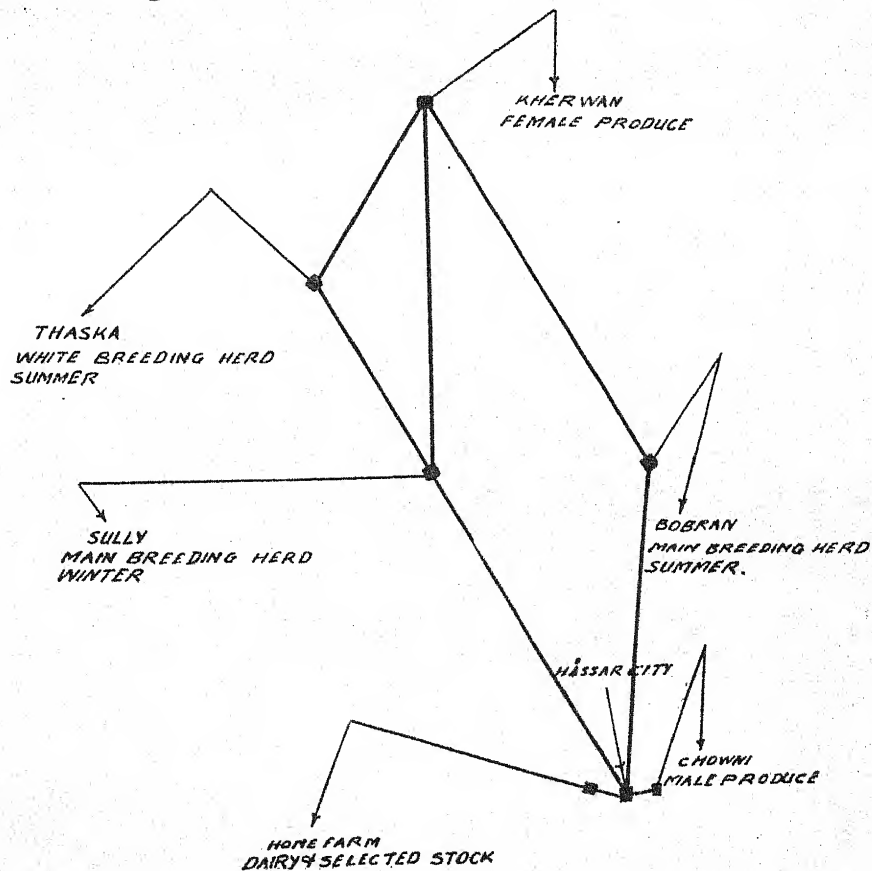
Fig. I.

HOUSING ARRANGEMENT OF CATTLE OF THE GOVERNMENT CATTLE FARM, HISSAR.

Strength of the stock.—The following will give some idea of the strength of the stock on the farm which were counted on the 25th February, 1935 :—

Cows	2,792
Male produce	1,684
Female produce	1,730
Herd Bulls	54
Supernumerary bulls	11
Castrated produce	49
Bullocks	464
Total	6,784

HISTORY OF THE OUTBREAK

A heifer was brought for treatment from Kherwan to the Farm Veterinary Hospital, situated in the Home Farm, on the 27th June, 1934. This animal manifested marked depression and dullness. The ears were drooping and the back arched. The muzzle was dry and the skin lustreless. Rumination was absent and the respiration accelerated. The next day tears were running profusely from the eyes. There was only a slight elevation of the body temperature. The bowels were constipated.

Two or three days after, small protuberances and vesicles developed on the neck, before and behind the shoulders, along the vertibral column and in the flank. In the mouth, especially on the mucous membranes of the lips, gums, and under the tongue some irregular erosions were seen but there was nothing characteristic about them.

The next day the animal died and on making a *post mortem* examination, the disease was confirmed as Rinderpest.

In the meantime, the disease at Kherwan flared up and a few more animals began to manifest symptoms. But the entire smyptom-complex was only exceptionally present in a single animal. In the Kherwan herd in some animals the functional disturbances of the respiratory organs or those of the digestive were more in the fore-ground, while in others a simultaneous affection of all organs was present in varying intensity. In most cases the illness was of a very short duration and the body temperature showed a slight elevation.

In the great majority of these severe cases, the disease lasted on an average from four to seven days.

The disease was brought under control at Kherwan by the serum-alone method but within a fortnight or so it broke out in the Home Farm stock and also spread to cows and calves at Bobran. The source of infection was definitely traced to the villages which are situated in the close vicinity of the farm and use farm roads as means of communication. The total loss, so far, was 80 and the situation appeared to be very grave on account of the high susceptibility of the farm stock.

Edwards [1925], in his paper on ' Some recent advances in the protection of cattle and other animals against disease ', states as follows :—

" The theory of the ' waves ' of intensity in rinderpest would seem capable of explanation in a similar manner. After the onset of a ' wave ' of rinderpest the disease spreads with increasing intensity until the virus eventually finds that the amount of susceptible ' soil ' remaining, upon which it can become readily implanted, becomes more and more rare. The virus itself thus loses to a considerable degree its properties of penetrating in the animal body, and it is not until there is available in abundance a new susceptible ' soil ' represented by the cattle

progeny that have grown up in the interval that the 'virus' finds a medium at hand for its ready propagation and, concomitantly, progressive exaltation of its disease-producing properties."

This explanation of Edwards applied in a remarkable degree to the susceptibility of the farm stock which had remained free from this disease for a number of years.

According to Haupt it requires from 5 to 6 weeks before the disease affects all animals of a herd consisting of 15 to 20 animals. Consequently rinderpest did not spread very rapidly in large herds of the farm in the outbreak under report.

CONTROL OF THE OUTBREAK

Kerr and Menon [1934] report that goat blood virus alone and goat tissue vaccine alone was used successfully on an extended scale in face of the disease in Bengal, and similar conditions being the experience in this province, it was decided to control the outbreak by means of goat virus alone vaccination.

To commence with, it was decided to immunise the female produce at Kherwan which were then free from infection. These inoculations at Kherwan were meant to serve a dual purpose; firstly to immunise the animals which were becoming once more susceptible on account of the passing off of the serum effects and, secondly, to study the nature of the reactions to be expected in the main herd. Consequently 1,151 heifers and 33 bullocks were inoculated with the goat virus.

It was then decided to tackle the main infected herd where goat tissue vaccine was brought into use. Any animal exhibiting the slightest signs of ill-health was excluded from the vaccine inoculations and segregated for observation. In all, the following number of incontact animals were vaccinated :—

Bobran—
Cows and calves 1,750
Bulls 28
Bullocks 10

Sully—
Cows 267
Calves 142
Bullocks 66

Thaska—
Bullocks 62

Home Farm—
Cows and heifers 250
Bullocks 164

Mundianwala—
Bullocks 109

Stable—
Bullocks 69

Total 2,817

REACTIONS IN THE CASE OF BLOOD VIRUS

In some animals typical rinderpest reactions, following the injection of virus, were seen. In others graded reactions of the same type were witnessed, which in most cases consisted of fever only. All the animals subjected to goat virus were examined twice daily and, if found to be suffering from severe reaction, treatment was taken up immediately. In highly susceptible animals, there was a sharp rise in temperature, usually not later than the fifth day after inoculation, which persisted for 3 days and towards the end of this period the appearance of vesicles occured in the mouth.

The percentage of reactors, in this case, was found to be 75. The degree of febrile reaction was also studied and it was found that in case of blood virus, the reaction was more pronounced, the temperature ranging between 103 5° to 106°. These animals also showed a well-marked depression. Six heifers showed exceptionally severe reactions and had to be fed on succulent diet for a few days, but none needed any special medical attention.

REACTION IN THE CASE OF GOAT TISSUE VACCINE

In the case of goat tissue vaccine, the temperature did not rise beyond 104°, the majority of animals showing 103° to 103·5°. The percentage of reactors was only 50. There was hardly any depression in these animals.

TEMPERATURE RECORDING

The temperature recording was carried out in the following manner. Out of each batch of animals inoculated during the course of a day, 20 animals were selected at random and their temperature was recorded on the 4th, 5th, and 6th day after the inoculations. In this way, 40 blood inoculated and 80 goat tissue vaccinated animals were brought under observation.

VACCINATION OF UNAFFECTED SEGREGATED ANIMALS

The male stock at Chowni was temporarily excluded from these inoculations as there appeared to be no immediate danger of the disease spreading to that area. On account of its natural segregated situation, there was every possibility of the place remaining free for at least a number of days, and so every attention was paid in this period to incontact vaccinated animals.

After the completion of work with the general herd the vaccine inoculation of the male produce was then undertaken. 1,173 male calves and 26 bullocks were vaccinated.

During the course of these inoculations a case of rinderpest occurred amongst the lot of bull calves that were still uninoculated. The incontact animals were immediately isolated and vaccinated with goat virus vaccine. Out of these incontacts, 6 more calves developed Rinderpest. Evidently these animals were in

the incubation stage of the disease at the time of vaccination. These were then subjected to serum treatment and cured.

The outbreak was brought under control within a period of 12 days with the loss of 18 animals from the time of vaccination or inoculation measures being undertaken.

Out of the cows and young calves at Thaska and Sully, which were in contact with the affected animals at the time of the outbreak, 80 animals developed rinderpest and were treated with anti-rinderpest serum (class P or II) in doses ranging from 100 c. c. to 250. In some cases these injections had to be repeated three or four times according to the condition of the patient.

The total number of animals inoculated with goat blood virus and goat tissue virus were :—

1. *Kherwan*—
   - Heifers 1,151
   - Bullocks 33

2. *Bobran*—
   - Cows and calves 1,750
   - Bulls 8
   - Bullocks 10

3. *Sully*—
   - Cows 267
   - Calves 142
   - Bullocks 66

4. *Thaska*—
   - Cows and calves 648
   - Bullocks 62

5. *Home Farm*—
   - Cows and heifers 250
   - Bullocks 64

6. *Mundianwala*—
   - Bullocks 109

7. *Stables*—
   - Bullocks 69

8. *Chowni*—
   - Bulls and bull calves 1,173
   - Bullocks 33

   Total 5,848

PREPARATION OF VACCINE

Both the blood and tissue vaccines employed at the farm for the immunisation of cattle were supplied by the Pathological Section of the Punjab Veterinary College, Lahore.

The attenuated goat virus employed at the Punjab Veterinary College for the purpose of manufacturing blood and tissue vaccine, was obtained from the Imperial Institute of Veterinary Research, Muktesar, about 2 years back and maintained by continuous passage in local bred goats.

The blood virus issued for use at the farm was 24 to 48 hours old. It was supplied in the form of whole blood mixed with equal parts of 1 per cent sodium citrate solution. The dose of the citrated solution of goat blood virus was 2 c. c. per head.

The goat tissue virus used at the farm was about 1 to 15 days old. It was despatched from the Lahore College in ampoules each containing one gramme piece of spleen tissue.

The maceration and dilution of vaccine was carried out at the farm according to the instructions issued by the Imperial Institute of Veterinary Research, Muktesar.

Each one gramme piece of spleen was macerated in 100 c.c. of normal saline solution and used in 1 c. c. dose per animal. The blood and tissue vaccines were despatched from the College, per passenger train, packed in ice and saw dust.

During the course of these inoculations it was sometimes necessary to keep a quantity of tissue vaccine over-night. In such instances the material was kept in a cool dark place packed in a large quantity of ice.

CHOICE OF THE VIRUS

The choice between the two types of virus is very difficult. It is not yet definitely ascertained that a febrile reaction is necessary for conferring lasting immunity. But, if immunity is assured only when the animal body reacts to the vaccine, then there is no doubt that blood vaccine is definitely better than the tissue vaccine. If, however, this is not essential, then there is no doubt that the tissue vaccine is to be preferred in the field inoculation work. It is easy of application in the field, its cost of production is extremely low ; its distribution is inexpensive, and its longer viability in storage is its greatest outstanding feature.

Kerr and Menon [1934] in their note regarding the use of goat virus as a means of controlling rinderpest outbreaks observe that goat blood vaccine was not suitable for general application in the field as it necessitated the presence of more experienced officers to control the operations. Besides, it was impracticable to maintain at headquarters, a large stock of potent goat blood vaccine. On the other hand, the goat tissue vaccine was easily prepared and preserved in sealed ampoules which were quite handy for packing and despatch by post. Moreover,

one goat produces 2,000 to 2,500 doses of tissue vaccine as compared with some 500 doses of blood virus. So far our experience coincides with the above workers of Bengal regarding the utility and use of the goat blood virus and the goat tissue vaccine.

CONCLUSIONS

We may sum up the position very briefly by saying that means are now abundantly to hand for combating natural outbreaks of rinderpest in the conditions in which they prevail in the Punjab. The goat virus method has now reached a state of perfection when no obstacle need reasonably be placed against its adoption on the grounds of excessive risk. Edwards [1929] in his address on ' The problems of Rinderpest in India ' read by him at the Conference of the Central Provinces Veterinary Association, held at Nagpur, November 1928, remarked that the system of protection most suitable for adoption in India is one of widespread vaccination and we entirely endorse his views on the subject.

The authors desire to record that Mr. S. M. A. Shah, the Farm's Senior Veterinary Officer, was in charge at the time of the outbreak of this disease, but owing to his transfer subsequently he was unable to collaborate in the writing of this article.

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A NOTE ON OSTEITIS DEFORMANS IN TWO FOWLS

BY

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FOREWORD

Several bony diseases affect poultry. These may be either neoplastic, inflammatory, or constitutional in their origin. Though pathological conditions occur commonly in bones, yet very little is understood regarding their nature. The technical difficulties associated with their histological examination are many and the introduction of such terms as calcareous infiltration, calcification, ossification, exostosis, osteitis, osteo-sclerosis, hyperostosis and the like merely convey an idea of the nature of such changes.

Diseases occurring in bones go by different names depending upon their origin, situation, and histological characters. When neoplastic in origin, they may grow out as exostoses in the form of excrescenes from the outer surface of a bone or may grow in and project into the medullary cavity. In some instances, the matrix becomes dense resulting in ' compact or ivory osteoma '. The majority of the osseous growths appear to be inflammatory in origin, as for example the formation of a callus round a fracture. Sometimes, the infiltration of chronic inflammatory foci with calcium salts may simulate bone formation and it must be remembered that the nature of the majority of bone tumours may be very difficult to interpret.

Diseases of constitutional origin also affect the skeletal system in poultry. These are all grouped under nutritional or vitamin deficiency diseases such as asthenia (going light), gout, rickets, osteoporosis, osteomalacia and osteitis deformans. Osteitis deformans is the name applied to the morbid process which usually affects the entire skeleton in adult life. It is a chronic constitutional disease characterised by the deposition of a fibro-osteoid tissue in such an excess as to enlarge and harden the affected bones. This kind of chronic osteitis is of a *formative* type, in which there is new formation of bony tissue from the osteogenic layer of the periosteum and sometimes also from the endo-osteum in the medullary canal giving the bone an ivory like consistency ; the other kind is known as the *rarefying* type in which there is absorption of bone, which is replaced by granulation tissue, as in tuberculosis and actinomycosis of the jaw in cattle.

This note is intended to record briefly an unusual condition of the limbs seen in two fowls. The cases are of further interest as they revealed spirochaetes in their blood in addition.

SUBJECTS.   In the month of August 1934, a condition quite analogous to the one described by Kaupp [1933] under " Osteitis deformans " was met with in two adult leghorn fowls. Both the fowls developed a striking enlargement of the legs which attracted the attention of the owner. The fowls, a cock and a hen, were brought to the Veterinary College Hospital for observation and treat-ment. (Plates II & III, Fig. 1).

SYMPTOMS.   In both the fowls, the lesions appeared to be one of Osteitis affecting the long bones, *viz.*—the tibia and the metatarsus. Enlargement and diffuse thickening of the bones were felt on manipulation. A skiagram of the limbs of these birds was taken for study and it revealed a condition of sclerosis. (Plate II, Figs. 2 & 3 and Plate III, Fig. 2.)

The cock showed more prominent lesions than the hen. Both the fowls appeared dull and inactive. They were drowsy and not feeding properly. The cock was passing yellowish white stools. The temperature of the hen ranged from 106° to 109° F.

CLINICAL OBSERVATION AND TREATMENT.   Dark ground illumination of the blood from the two fowls revealed spirochaetes and they were noticed to be in larger numbers in the cock. This finding of spirochaetes associated with such unusual lesions in the fowls suggested the possibility of a condition simulating gummatous osteitis, caused by syphilis in man, by an analogous organism. But such a condition in fowls, as far as can be ascertained from the literature on spiro-chaetosis, has not been recorded. Spirochaetes usually cause an acute infection and rapid termination, hence it is obvious that they may have not been the cause of such lesions as are described in this paper. The fowls were treated with soamin for spirochaetosis. The hen was destroyed after 27 days and during this period, the thickness of the legs was found to have slightly increased.

AUTOPSY.   *Post mortem* examination was held on both the fowls. The cock was emaciated and revealed a considerable enlargement and hardness of both the tibia and metatarsal bones. To see the extent of damage, one of the bones was sawn through longitudinally and it showed dense compact tissue almost ivory like, filling the medullary canal. (Plate III, Fig. 3-a, longitudinal section.) Fatty changes were noticed in the liver. Smears from the heart blood revealed spirochaetes.

The hen, on *post mortem* examination, also presented an anaemic and emaciated appearance.

The bones, tibia and metatarsus, on longitudinal section, showed deposition of compact tissue, but to a lesser degree. The remains of the cancellated tissue could be seen partially in the metatarsus. (Plate III, Fig. 3-b.) A similar section of metatarsus from a normal fowl is placed for comparison in the photograph appended. (Plate III, Fig. 3-c.) No pathogenic organisms were present in the smears from the bone marrow.

DISCUSSION. The condition described above should be differentiated from scaly leg and productive inflammation of the soft structures of the shank of fowls, which also develop without any signs of acute inflammation. The shank, in the latter affection, gradually becomes larger due to newly formed connective tissue, but the increasing enlargement is limited to the soft structures alone, the bony tissue not being involved. A skiagram will reveal its true nature.

The condition of chronic osteitis may either be a sequel of certain constitutional disturbances such as " Osteitis deformans " or ' rickets ', or it may result from chronic bacterial infections such as tuberculosis, actinomycosis and gummatous osteitis.

As regards the causation of Osteitis deformans in man, various theories have been proposed and many infectious agents have been held responsible including the organism of syphilis. It was held, by some, to be due to the effect of the disturbance of some internal secretion. There appears to be little evidence in support of these views. MacCallum [1924] refers to this condition as " Paget's disease " and, in 1876, he described it in human beings as affecting the bones of the extremities and the skull. It is further observed that the changes in some cases may be unilateral or may be limited to one or two bones. The condition progresses slowly with some tenderness and pain in the altered bones. The marrow loses its blood forming elements and becomes converted into a vascular fibrous tissue which produces much soft bone-like tissue ; the marrow cavity is encroached upon and filled completely. The softened bone later assumes an ivory-like hardness. So Paget inclines to the view that the process is of an inflammatory nature, resulting in enlargement and excessive production of imperfectly developed osseous tissue which becomes organised.

MacCallum [1924] in dealing with gummatous osteitis, describes a form in which the new production of bone is more extensive than in the periosteal type and states that it is deposited in each Haversian system and through the cancellous bone in the interior so that the shaft of the bone becomes dense and ivory-like and the whole bone is much heavier than normal.

Kaupp [1933] has described the condition of ' Osteitis deformans ' as occurring in fowls and observes that Goldman has described a case in fowls affecting the front part of the skull and the long bones, including the femur, humerus and metatarsus. Many have held that rickets, osteomalacia and osteitis deformans are manifestations of the same disease bearing on impaired nutrition and deranged matabolism. It is supposed that, in the course of calcification, a certain amount of the sulphur of the matrix is replaced by other elements, which must entail the retention of calcium, magnesium and phosphorus and involve the increased elimination of sulphur.

Kaupp states that DaCosta and his co-workers interpret the retention of calcium, magnesium and phosphorus with excessive excretion of sulphur found
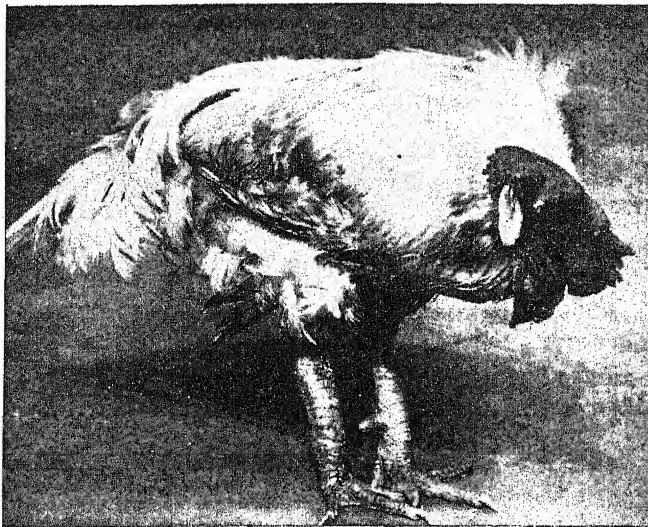
FIG. 1

FIG. 1. Leghorn Cock—Note the dull posture and drooping of the head and tail (spirochaete infection), with enlargement of the bones of the metatarsus (both legs.)

FIG. 2　　　　　　FIG. 3

FIG. 2. Skiagram revealing the thickened condition of the bone in the cock.

FIG. 3. Skiagram (for comparison) of the limb of a normal fowl revealing no alteration in the bones.
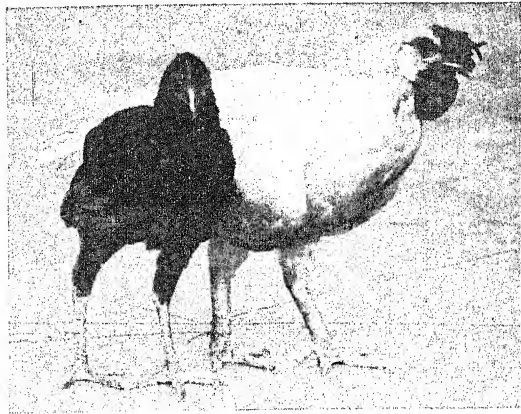
Fig. 1

Fig. 1.   Leghorn hen and a normal hen for comparison.   Note the difference in the condition of the legs in both.

a   b   c

Fig. 2   Fig. 3

Fig. 2.   Skiagram revealing thickened condition of the bone in the hen.
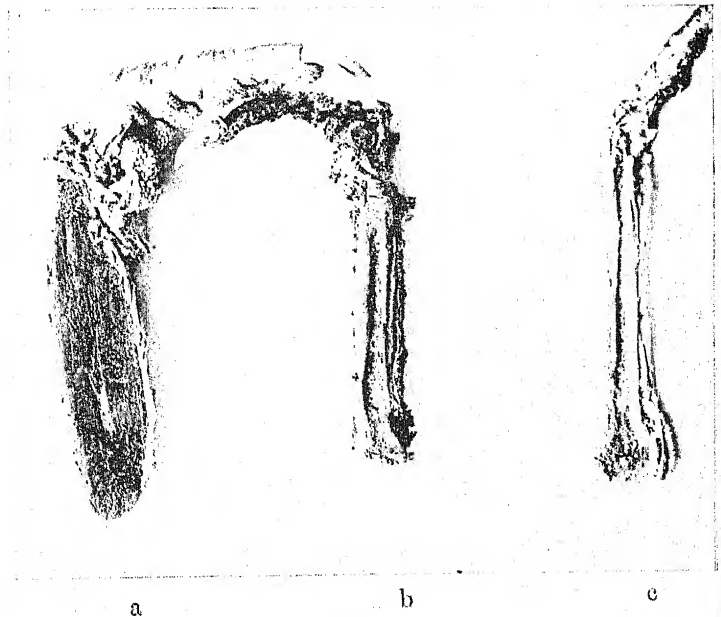
Fig. 3.   Longitudinal sections of the metatarsus of the two affected fowls ; a similar section of the metatarsus of a normal fowl is included for comparison.

(a) Cock—Note the uniform ingrowth of bony tissue into the medullary canal almost completely effacing and occluding it.

(b) Hen—This condition is partially seen in the hen.

(c) Normal fowl—Section of medullary canal of a normal fowl.   Note the uniform depth of the canal.

in these cases, as indicating a stimulated osseous or osteoid formation accompany
ing the resorption of a highly sulphurised organic matrix.

I wish to express my gratitude to Mr. T. J. Hurley, Principal, Madras Veteri-
nary College, for giving me an opportunity to study such lesions ; to Mr. M. Anant
Narayan Rao, Officer-in-charge, Laboratory for valuable suggestions and affording
facilities for writing on the subject ; to Mr. V. Janakirama Aiyar for lending the
use of the skiagrams, and to the Artist, Mr. P. Dorasamy Mudaliar, for the photo-
graphs and copies of the skiagrams.

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    Philadelphia and London.

ABSTRACTS

Deficient feed makes deficient milk. A. G. INGHAM. (*Hoard's Dairyman—August 25, 1935, 80, No. 16, page 391.*)

The simple fact is that milk is no better than the feed which the cow lives on. If the minerals and vitamins are not in the cow's feed they will not be in the milk.

Experiments clearly indicate that where cows are not being fed a ration of high quality natural feeds, the milk they produce will not bring about normal growth in their offspring. Milk thus produced on a deficient ration will be deficient milk and should be guarded against by the breeder who is trying to develop first class breeding stock. Would it not appear that such deficient milk should also be guarded against by the average milk producer as a protection to the ultimate consumer? This is a subject that it behoves the public health authorities to look into also.

If better feeding were practised along with better breeding, there would be less need for weeding. (C. E. M.).

Factors affecting economical manufacture, uniformity in composition and quality of butter. D. H. NELSON. (*Dairy Science, 18, No. 4, April, 1935.*)

The author deals with the factors influencing the economical manufacture and the uniformity in composition and quality of butter.

Such factors include the efficiency and conscientiousness of the employees, the efficiency of the machines used—but probably one of the most important factors is the overrun. The overrun reflects, in a large measure, all the other factors which affect both economical manufacture and uniformity of product. The butter manufacturer readily learns that other factors being equal, an increase in the overrun reduces the cost of manufacture and increases his profits. The butter maker usually controls his overrun by the moisture tester, and although the moisture in the butter is an important factor in the eventual overrun, this test does not give due credit to the salt content and curd content—both of them being as important in the control of overrun as is the moisture content.

In order to control his overrun, so that the fat content of butter will always fall between 80-81 per cent, the writer recommends that the butter manufacturer should use the Kohman Method for analysing each churning of butter. (C. E. M.).

ERRATA.

The Indian Journal of Veterinary Science and Animal Husbandry, Vol. V, part 3.

Pages 256—258. Wherever the sign ε occurs, substitute the sign Σ.

Page 261, lines 17 and 19. *For* $S/_1'^2$ and $S/_2'^2$ *read* $S_1 1^2$ and $S_2 1^2$.

Page 261, line 25. *For* B^{-kt} *read* Be^{-kt}.

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SELECTED ARTICLES

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SOME DIGESTIBILITY TRIALS ON INDIAN FEEDING STUFFS, PART X. GREEN FODDERS, HAYS AND GRAM *BHUSA*.

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Page 119,
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Page 187, The course of specific investigations on the feeding value of some
Healing' stuffs, plans of this series have been laid to investigate progressively
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Comma the more specific inquiries so far published. The results from a
miscellaneous materials grown at Lyallpur are given in this paper.

The fodders, the data from which are given in this paper, are shown in the following table, of which velvet beans, Elephant grass, berseem hay, and *juar* No. 11 were fed to Hissar bullocks and the remainder to mature dry Montgomery heifers.

1. *Senji* green.
2. Berseem green.
3. Velvet beans green.
4. Sunflower green.
5. *Bajra* green.
6. Elephant grass green.
7. Guinea grass green.
8. Sudan grass green.
9. *Juar* green.
10. *Juar* Nos. 8, 11 and 22 green.
11. Berseem hay.
12. Lyallpur Dub grass hay.
13. Gram *bhusa*.

ORIGINAL ARTICLES

SOME DIGESTIBILITY TRIALS ON INDIAN FEEDING STUFFS, PART X. GREEN FODDERS, HAYS AND GRAM *BHUSA.*

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(Received for publication on 25th February 1936.)

During the course of specific investigations on the feeding value of some Punjab feeding-stuffs, plans of this series have been laid to investigate progressively all the Punjab feeding-stuffs on the same principles and using the same technique as employed in the more specific inquiries so far published. The results from a number of such miscellaneous materials grown at Lyallpur are given in this paper.

The fodders, the data from which are given in this paper, are shown in the following table, of which velvet beans, Elephant grass, berseem hay, and *juar* No. 11 were fed to Hissar bullocks and the remainder to mature dry Montgomery heifers.

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11. Berseem hay.
12. Lyallpur Dub grass hay.
13. Gram *bhusa.*

Table I shows the complete chemical composition of the fodders, and the digestibility data are given in Table II. Starch equivalents, digestible protein per 100 parts of fodder, albuminoid ratios and the daily nitrogen balance are given in Table III, while the comparative feeding values of all the fodders are summarised in averages in Table IV.

Senji (Mililotus parviflora).—This is a *rabi* fodder available for feeding in the Punjab, by about the middle of January. The sample used was grown on a heavy loam soil and fed at the end of February in a green condition. *Senji* is very rich in highly digestible protein and proved to be a maintenance ration. The animals invariably showed a very high positive daily nitrogen balance which is reflected to some degree in a corresponding increase in the body weights during the trials. *Senji* is conspicuously rich in calcium.

Berseem (Trifolium Alexandrinum)—This is also a *rabi* fodder which yields about five cuttings in a season and is available for feeding by Christmas. The sample used was grown on a medium loam and was fed both in the green condition and as hay. The green fodder was obtained from the 3rd cut in March 1931, whilst the hay was made from a homogenous mixture of five cuttings obtained at intervals during the season. Green berseem is a maintenance ration, richer in calcium and phosphoric acid than green *senji*, but the digestibility of the protein of each of these green fodders is of the same order. The daily nitrogen balance was positive in both but somewhat lower in the case of berseem, a fact probably due to the smaller intake of the total dry matter by the animals when feeding on berseem. During the course of the trials extending over 22 days the animals increased in body weight. Reference to the figures in the last two columns of Table IV shows that green berseem is a decidedly better fodder than green *senji*.

Velvet Beans (Stizolobium deeringianum).—This bean is a *kharif* legume and was fed at the dough stage when it was found to be a maintenance ration; it compares favourably with green *senji* and green berseem in its protein content, and is decidedly richer than either in its calcium and phosphoric acid contents. The high daily nitrogen balance found in these trials with this fodder was reflected in a corresponding increase in the body weights of the animals.

Sunflower (Helianthus annuus).—This has recently been introduced in the Punjab as a ration for cattle and produces a heavy crop of succulent green fodder [Read, 1933]. It was fed in our trials at the "Milk-dough" stage and proved to be a maintenance ration comparing favourably with leguminous fodders.

Bajra. (Pennisetum typhoideum).—This *kharif* fodder was obtained from a light soil and fed at the "Milk-dough" stage and proved to be a maintenance ration.

Elephant grass (Pennisetum purpureum).—This is a perennial grass which grows luxuriantly and becomes hard and fibrous and unsuitable as a fodder after a stage when it is about three feet high.

The sample used in these trials was grown on a medium soil and cut at this stage and proved to be a maintenance ration.

Guinea grass (Panicum maximum)—This grass yields about six cuttings during the *kharif* season under irrigated conditions at Lyallpur and the sample used was the fifth cut representing the best stage of growth, *i.e.*, when about three feet high and proved to be a maintenance ration.

Sudan grass (Andropogon Sorghum Var : Sudanensis).—This is a *kharif* fodder which yields about four cuttings during the season. It was fed when in a condition a little past the 'Milk stage' of the final cutting but did not prove to be a maintenance ration.

Juar (Chari) local Sorghum Vulgare.—This is a *kharif* fodder commonly used for feeding cattle both in a green condition and as hay. The sample used in these trials was grown on a medium loam soil, fed in the pre-milk stage and was found to be a non-maintenance ration.

Juar (Chari) Nos. 8, 11 and 22.—These samples represent certain new Punjab *juars* obtained by crossing various sweet varieties. These were grown on a light soil and collected for these trials after the heads had been gathered for grain ; of the three varieties, only No. 8 which is the richest in protein proved to be a maintenance ration.

Lyallpur Dub grass hay (Cynodon dactylon).—This hay was made from lawn mowings from the College estate, collected over a period of about eight months. Each cut was dried in the sun and a composite sample of the mixture, which proved to be a maintenance ration, was used in the trials. This hay is rich in protein.

Gram bhusa.—This is a chaff of a *rabi* leguminous crop *Cicer arietinum*. It is richer in digestible nutrients than wheat *bhusa* [Lander and Dharmani, 1932] and like wheat *bhusa*, varies in composition from year to year but, nevertheless, was not a maintenance ration.

It is usual to find small differences in chemical composition in the same type of fodder when grown under different climatic conditions or on different types of soil but the analytical data given here may be taken as representative of these fodders as grown in the Punjab at the particular stage of growth indicated.

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TABLE 1

Chemical Composition

Name of the Feed	Moisture per cent	Dry Matter per cent	Ash per cent	Fat per cent	Fibre per cent	Protein per cent	Nitrogen-free extract per cent
1. *Senji* green	78·40	21·60	2·82	0·36	6·35	3·61	8·70
2. Berseem green	84·90	15·10	2·36	0·42	2·94	2·75	4·63
3. Velvet Beans green	76·02	23·98	3·58	0·51	4·62	3·65	11·64
4. Sunflower green	79·06	20·94	3·22	0·72	5·02	2·50	9·48
5. *Bajra* green	78·36	21·64	2·35	0·33	6·89	1·59	10·57
6. Elephant grass green	77·83	22·17	3·12	0·45	7·39	1·40	10·81
7. Guinea grass green	74·90	25·10	3·65	0·59	9·13	1·31	11·42
8. Sudan grass green	61·64	35·36	3·61	0·57	11·31	1·44	18·46
9. *Juar* green	67·02	32·98	2·88	0·48	11·48	1·43	17·81
10. *Juar* (Chari) No. 8 green	60·25	39·75	3·76	0·67	10·80	1·94	22·58
11. *Juar* (Chari) No. 11 green	61·02	38·98	3·76	0·54	10·52	1·44	22·72
12. *Juar* (Chari) No. 22 green	61·40	38·60	2·47	0·46	10·70	0·85	24·12
13. Berseem hay	12·77	87·23	12·57	1·03	18·53	13·68	41·52
14. Lyallpur *Dub* grass hay	8·73	91·27	11·45	1·26	16·78	19·13	51·66
15. Gram *bhusa*	9·40	90·60	12·06	0·45	40·27	5·44	32·35
16. Wheat *bhusa*	7·60	92·40	9·43	0·88	38·04	2·19	40·26

Chemical Composition. Per cent on oven-dried fodder.

Name of the Feed	Moisture per cent	Dry Matter per cent	Ash per cent	Fat per cent	Fibre per cent	Protein per cent	Nitrogen-free extract per cent
1. *Senji* green	…	…	13·06	1·67	29·40	15·33	40·54
2. Berseem green	…	…	15·63	2·78	19·47	18·21	43·91
3. Velvet Beans green	…	…	14·93	2·13	19·27	15·14	48·53
4. Sunflower green	…	…	15·38	3·44	23·98	11·04	45·26
5. *Bajra* green	…	…	10·86	1·52	31·84	6·93	48·85

6. Elephant grass green	14·08	2·03	33·84	4·51	46·04
7. Guinea grass green	12·15	1·55	36·38	5·22	44·70
8. Sudan grass green	10·21	1·61	31·99	4·07	52·12
9. Juar green	8·73	1·46	34·82	3·43	51·56
10. Juar (Chari) No. 8 green	9·46	1·69	27·17	4·88	56·80
11. Juar (Chari) No. 11 green	9·65	1·39	26·99	3·70	58·27
12. Juar (Chari) No. 22 green	6·40	1·19	27·72	2·20	62·49
13. Berseem hay	14·07	1·18	21·25	15·91	47·59
14. Lyallpur Dub grass hay	12·54	1·38	18·38	11·10	56·60
15. Gram bhusa	13·31	0·53	44·45	6·01	35·70
16. Wheat bhusa	10·21	0·95	42·15	2·37	44·32

B

TABLE I—contd.

Mineral matter in 100 lbs. of the oven-dried feed

Name of feed	Phosphates P_2O_5	Calcium CaO	Sodium Na_2O	Potassium K_2O	Magnesium MgO	Manganese Mn_3O_4	Aluminium Al_2O_3	Iron Fe_2O_3	Sulphate SO_4	Insoluble Residue	Iodine in (Y)= 0·00001 grms.
1. *Sengi* green	0·437	1·440	0·149	3·50	0·515	0·0100	0·469	0·135	0·560	3·13	25·6
2. Berseem green	0·440	2·660	0·257	4·77	0·549	0·0105	0·343	0·106	0·365	2·14	35·0
3. Velvet beans green	0·753	4·080	0·218	2·87	1·060	0·0153	0·503	0·161	0·399	3·20	38·5
4. Sunflower green	0·505	2·460	0·086	4·25	1·220	0·0103	0·378	0·181	0·251	3·51	20·0
5. *Bajra* green	0·303	0·760	0·324	3·50	0·543	0·0083	0·190	0·065	0·798	4·27	21·6
6. Elephant grass green	0·700	0·775	0·612	4·41	0·451	0·0067	0·368	0·117	0·410	5·55	16·0
7. Guinea grass green	0·629	0·749	0·200	3·23	0·603	0·0055	0·160	0·051	0·198	5·45	29·7
8. Sudan grass green	0·434	0·927	0·004	1·54	0·687	0·0101	0·240	0·124	0·241	5·80	16·0
9. *Juar* green	0·208	0·674	0·158	2·41	0·253	0·0078	0·378	0·000	0·122	4·42	21·0
10. *Juar* No. 8 green	0·219	0·937	0·076	2·08	0·398	0·0067	0·259	0·089	0·185	4·08	14·0
11. *Juar* No. 11 green	0·194	0·703	0·096	2·47	0·383	0·0112	0·341	0·005	0·188	5·10	18·0
12. *Juar* No. 22 green	0·183	0·645	0·100	2·04	0·330	0·0000	0·323	0·007	0·133	4·03	15·0
13. Berseem hay	0·352	2·580	0·446	4·49	0·679	0·0123	0·702	0·166	0·443	4·29	32·6
14. Lyallpur *Dub* grass hay	0·557	1·130	0·119	2·12	0·415	0·0101	0·452	0·115	0·760	6·26	25·0
15. Gram *bhusa*	0·167	1·160	0·639	2·98	0·830	0·0046	0·151	0·056	0·425	1·02	19·7
16. Wheat *bhusa*	0·060	0·358	0·161	1·75	0·151	0·0420	0·164	0·141	0·458	5·26	..

TABLE II

Digestibility Data

Period	Animal	Daily body weight lbs.	Ration	Feed eaten per day lbs.	Digestibility coefficients — Dry matter	Ash	Fat	Fibre	Protein	Nitrogen free extract	Digestible constituents per 100 lbs. of feed — Dry matter	Ash	Fat	Fibre	Protein	Nitrogen free extract
29th Feb. 1932 to 9th March 1932.	128	690	*Senji* green	84·1	68·78	55·28	46·07	57·68	82·73	76·80	14·85	1·56	0·17	3·66	2·74	6·72
	129	725	Do.	85·4	69·70	60·17	48·89	59·77	83·04	75·67	15·06	1·70	0·18	3·79	2·76	6·68
	133	595	Do.	66·0	65·36	46·24	33·33	56·08	79·00	74·39	14·12	1·30	0·12	3·56	2·62	6·52
8th March 1931 to 20th March 1931.	128	574	Berseem green	77·9	72·10	61·41	48·48	59·39	80·84	79·50	10·89	1·54	0·20	1·75	2·22	5·27
	129	568	Do.	82·3	73·20	62·37	51·43	60·74	82·30	80·22	11·05	1·47	0·22	1·79	2·26	5·32
	133	451	Do.	67·6	72·07	61·87	50·00	61·30	80·65	80·35	11·02	1·46	0·21	1·80	2·22	5·33
23rd Nov. 1933 to 29th Nov. 1933	Bu. 2	950	Velvet Beans green	70·0	68·26	40·23	66·67	59·31	69·96	79·90	16·37	1·44	0·34	2·74	2·54	9·30
	Bu. 3	1,052	Do.	70·0	64·26	35·48	58·83	49·79	66·94	78·28	15·41	1·27	0·50	2·30	2·43	9·11
	Bu. 4	865	Do.	70·0	66·67	37·99	60·79	57·58	68·60	78·80	15·99	1·36	0·31	2·66	2·49	9·17
	Bu. 4		Do.	70·0	73·93	51·13	70·60	68·40	76·02	82·74	17·73	1·83	0·36	3·10	2·76	9·63
9th Oct. 1934 to 20th Oct. 1934.	G. 2	830	Sunflower green	73·5	53·43	40·68	41·66	17·93	70·80	72·90	11·19	1·31	0·30	0·90	1·77	6·91
	S. 1	506	Do.	48·6	54·24	48·45	45·83	22·51	72·40	68·88	11·36	1·56	0·33	1·13	1·81	6·53
29th Aug 1935 to 8th Sept. 1935	166	840	*Bajra* green	62·4	61·40	51·07	66·66	55·15	62·00	67·55	13·29	1·20	0·22	3·80	0·93	7·14
	G. 2	883	Do.	62·1	63·63	47·24	63·64	61·24	59·33	69·45	13·77	1·11	0·21	4·22	0·89	7·34
	S. 1	574	Do.	48·0	65·48	56·66	69·70	62·84	64·00	69·26	14·77	1·33	0·23	4·33	0·96	7·32
28th Sept. 1932 to 12th Oct. 1932	Bu. 1	870	Elephant grass green	70·9	62·86	52·97	56·25	66·22	63·88	63·53	13·03	1·65	0·25	4·89	0·63	6·49
	Bu. 4	850	Do.	65·3	61·33	55·42	62·04	63·54	60·00	61·59	13·60	1·73	0·28	4·70	0·60	6·29
	Bu. 2	1,000	Do.	74·5	62·41	54·74	58·82	60·43	64·00	60·12	13·83	1·71	0·27	4·47	0·64	7·06
7th Sept. 1931 to 18th Sept. 1931	149	570	Guinea grass green	53·5	56·73	42·95	43·59	57·27	60·31	60·09	14·24	1·31	0·17	5·23	0·79	6·74
	151	530	Do.	38·4	57·36	43·60	41·06	58·69	59·55	60·35	14·40	1·32	0·16	5·36	0·73	6·77
	156	520	Do.	37·1	55·29	37·04	41·03	57·27	58·02	58·88	13·88	1·13	0·16	5·23	0·76	6·60
7th Sept. 1931 to 18th Sept. 1931	128	720	Sudan grass green	29·5	45·54	...	29·41	57·78	23·25	49·64	16·10	...	0·17	6·54	0·34	9·15
	129	710	Do.	28·3	45·40	...	31·25	54·06	29·27	50·77	16·06	...	0·18	6·11	0·42	9·36
	133	600	Do.	25·0	48·54	...	35·71	61·88	30·55	51·42	17·17	...	0·20	6·99	0·44	9·48

Table II—contd.

Period	Animal	Daily body weight lbs	Ration	Feed eaten per day lbs.	Digestibility coefficients						Digestible constituents per 100 lbs. of feed					
					Dry matter	Ash	Fat	Fibre	Protein	Nitrogen free extract	Dry matter	Ash	Fat	Fibre	Protein	Nitrogen free extract
6th Sept. 1930 to 11th Sept. 1930	128	584	Juar (Chari) green	30·5	56·76	12·50	33·33	65·14	40·00	60·50	18·72	0·36	0·16	7·48	0·46	10·29
	129	542	Do.	26·0	54·40	29·33	46·15	60·20	37·93	64·05	19·28	0·35	0·12	6·91	0·42	10·89
	133	451	Do.	22·5	53·77	10·92	39·36	57·75	32·00	59·27	17·76	0·49	0·18	6·63	0·36	10·05
21st Nov. 1932 to 28th Nov. 1932	160	454	Juar (Chari) No. 8 green	17·1	57·05	18·75	41·67	60·55	42·42	63·47	22·68	0·71	0·25	6·54	0·82	14·33
	171	440	Do.	17·6	56·00	17·18	38·85	60·52	41·15	61·71	22·25	0·68	0·22	6·51	0·80	13·03
	172	420	Do.	18·2	57·26	…	50·40	59·33	45·70	59·61	22·76	…	0·34	6·41	0·88	13·46
21st Nov. 1932 to 28th Nov. 1932	Bu. 1	820	Juar (Chari) No. 11 green	26·0	51·78	10·20	26·57	55·47	21·00	59·39	20·19	0·80	0·15	5·84	0·31	13·50
	Bu. 2	980	Do.	30·6	50·18	10·44	35·30	51·56	22·75	58·27	19·54	0·39	0·19	5·42	0·33	12·24
10th Dec. 1932 to 18th Dec. 1932	169	498	Juar (Chari) No. 22 green	13·8	52·81	−23·00	7·81	56·77	−16·07	63·47	20·39	Negative	0·04	6·07	Negative	15·81
	171	422	Do.	12·0	58·31	−13·00	16·47	61·00	−0·69	67·30	22·51	"	0·08	6·59	"	16·23
	172	410	Do.	14·3	52·18	−23·00	14·29	58·60	−25·00	62·70	20·14	"	0·07	6·73	"	15·14
1st Feb. 1934 to 16th Feb. 1934	Bu. 1	1,050	Berseem hay	18·0	64·14	41·25	33·04	48·84	68·02	77·30	55·04	5·66	0·32	9·05	9·05	32·05
	Bu. 2	1,100	Do.	17·9	62·46	57·84	81·47	46·41	68·08	75·62	54·48	4·64	0·22	8·09	8·94	31·40
	Bu. 3	1,080	Do.	18·5	66·50	44·20	31·47	51·45	71·62	76·02	57·18	5·30	0·33	8·57	9·94	31·04
	Bu. 4	980	Do.	15·2	65·04	45·50	32·65	48·80	71·54	70·66	56·74	5·02	0·33	8·05	9·95	31·88
3rd Jan. 1934 to 12th Jan. 1934	169	640	Iyalpur Dub grass hay	18·1	42·62	16·63	21·61	58·28	52·62	41·57	38·62	1·91	0·81	9·77	5·27	21·57
	171	615	Do.	19·9	50·15	15·20	25·37	58·55	57·94	54·17	45·78	1·72	0·27	9·82	5·87	27·08
	G. 1	565	Do.	11·8	41·01	17·03	25·73	42·47	58·59	43·69	37·44	1·65	0·35	7·17	5·40	22·57
19th Jan. 1933 to 25th Jan. 1933	160	463	Gram bhusa	8·1	44·14	34·70	…	43·25	46·95	49·62	38·90	4·19	…	17·42	2·20	16·06
	171	461	Do.	7·9	40·22	35·70	…	37·30	37·24	46·10	39·44	3·60	…	14·94	2·68	14·91
	172	420	Do.	7·4	40·39	30·34	…	39·91	42·50	49·92	37·14	…	…	16·60	2·30	14·89
5th Aug. 1929 to 14th Aug. 1929	122	469	Wheat bhusa	6·4	40·70	…	33·33	59·04	…	50·38	46·13	…	0·31	22·97	Negative	20·63
	128	450	Do.	6·3	52·24	…	33·33	65·21	…	55·04	48·26	…	0·32	25·40	"	22·54
	133	357	Do.	5·3	47·19	…	40·00	59·11	…	52·12	48·60	…	0·39	23·08	"	21·85

TABLE III

Food Values

Period	Animal	Daily Body weight lbs.	Name of the feed	Daily Nitrogen balance grms.	Starch equivalents	Digestible protein per 100 lbs. of the feed	Albuminoid ratio 1:	Remarks.
25th February 1932 to 9th March 1932.	128	690	Senji green	72·74	11·10	2·74	3·9	Maintenance Ration.
	129	725	Do.	75·56	11·17	2·76	3·9	Ditto
	133	595	Do.	51·53	10·69	2·62	4·0	Ditto
8th March 1931 to 20th March 1931	123	574	Berseem green	55·13	8·71	2·22	3·4	Ditto
	129	568	Ditto	67·53	8·86	2·26	3·4	Ditto
	133	451	Ditto	52·59	8·82	2·22	3·4	Ditto
23rd Nov. 1933 to 29th Nov. 1933	Bu.1	950	Velvet Beans green	35·17	13·74	2·54	5·1	Ditto
	Bu.2	1,052	Ditto	13·50	12·92	2·43	5·0	Ditto
	Bu.3	935	Ditto	32·32	13·42	2·49	5·0	Ditto
	Bu.4	865	Ditto	45·37	14·75	2·76	4·9	Ditto
9th Oct. 1934 to 20th Oct. 1934	G.2	820	Sunflower green	21·00	8·53	1·77	4·8	Ditto
	S.1	506	Ditto	14·40	8·48	1·81	4·7	Ditto
29th Aug. 1935 to 8th Sept. 1935	106	840	*Bajra* green	24·45	9·82	0·93	12·5	Ditto
	G.2	883	Ditto	28·53	10·38	0·89	13·5	Ditto
	S.1	574	Ditto	22·54	10·58	0·96	12·7	Ditto
28th Sept. 1932 to 12th Oct. 1932	Bu.1	870	Elephant grass green	19·57	9·58	0·63	18·8	Ditto
	Bu.4	850	Ditto	14·75	9·20	0·60	19·5	Ditto
	Bu.2	1,000	Ditto	20·63	9·45	0·64	18·4	Ditto
7th Sept. 1931 to 18th Sept. 1931	149	570	Guinea grass green	12·80	9·81	0·79	15·8	Ditto
	151	580	Ditto	8·19	9·41	0·78	14·7	Ditto
	156	520	Ditto	6·59	9·10	0·76	15·4	Ditto
7th Sept. 1931 to 18th Sept. 1931	128	720	Sudan grass green	−6·03	11·22	0·34	47·5	Non-maintenance Ration.
	129	710	Ditto	−4·47	11·13	0·42	37·5	Ditto
	133	600	Ditto	−3·95	12·20	0·44	35·5	Ditto

TABLE III—contd.

Period	Animal	Daily Body weight lbs.	Name of the feed	Daily Nitrogen balance grms.	Starch equivalents	Digestible protein per 100 lbs. of the feed	Albuminoid ratio 1:	Remarks
6th Sept. 1930 to 11th Sept. 1930	128	584	Juar green	+1·61	13·3	0·46	39·5	Ditto
	129	542	Ditto	+0·45	13·3	0·42	43·3	Maintenance Ration
	133	451	Ditto	+2·40	12·1	0·36	45·2	Non-maintenance Ration.
21st Nov. 1932 to 28th Nov. 1932	169	454	Juar No. 8 green	2·58	17·42	0·82	26·2	Maintenance Ration
	171	446	Ditto	2·54	16·34	0·80	28·4	Ditto
	172	420	Ditto	1·92	16·57	0·88	28·5	Ditto
21st Nov. 1932 to 28th Nov. 1932	Bu.1	529	Juar No. 11 green	4·94	15·34	0·81	64·0	Non-maintenance Ration.
	Bu.2	980	Ditto	5·09	14·74	0·93	58·5	Ditto
10th Dec. 1932 to 18th Dec. 1932	169	438	Juar No. 22 green	3·93	16·53	Negative	8 8 8	Ditto
	171	422	Ditto	6·50	18·12	,,		Ditto
	172	416	Ditto	8·01	16·15	,,		Ditto
1st Feb. 1934 to 16th Feb. 1934	Bu.1	1,050	Berseem hay	6·89	40·06	0·44	4·0	Maintenance Ration
	Bu.2	1,168	Ditto	11·06	38·76	0·55	4·22	Ditto
	Bu.3	1,090	Ditto	6·68	40·90	0·94	4·36	Ditto
	Bu.4	980	Ditto	9·33	40·10	0·93	4·2	Ditto
3rd Jan. 1934 to 12th Jan. 1934.	169	649	Lyallpur Deb grass hay	2·63	27·05	5·27	6·6	Ditto
	171	615	Ditto	0·57	34·35	5·57	6·6	Ditto
	6·1	565	Ditto	1·57	35·58	5·40	5·7	Ditto
19th Jan. 1933 to 25th Jan. 1933	169	468	Gram khusi	4·79	12·59	2·20	15·1	Non-maintenance Ration.
	171	461	Ditto	5·40	8·45	2·03	14·8	Ditto
	172	420	Ditto	4·39	10·65	2·30	12·6	Ditto
5th Aug. 1929 to 14th Aug. 1929	128	469	Wheat bhusa	0·45	21·43	Negative	8 8 8	Ditto
	129	429	Ditto	7·69	20·63	,,		Ditto
	133	357	Ditto	7·03	22·27	,,		Ditto

TABLE IV

Summary of data showing averages

Name of feed	Dry matter %	Digestibility coefficient.						Digestible constituents per 100 lbs. of the feed as such						Starch equivalent	Albuminoid ratio	Per cent on oven dried feed	
		Dry matter	Ash	Fat	Fibre	Protein	Nitrogen free extract	Dry matter	Ash	Fat	Fibre	Protein	Nitrogen free extract			Starch equivalents	Digestible protein
1. *Senji* green	21·60	69·05	53·89	42·79	57·84	81·50	75·62	14·08	1·52	0·16	3·67	2·71	6·62	10·96	3·9	50·74	12·55
2. Berseen green	15·10	72·76	61·88	49·97	60·43	81·26	80·02	10·99	1·46	0·21	1·78	2·23	5·31	8·80	3·4	58·28	14·78
3. Velvet beans green.	23·98	68·28	48·21	64·22	58·77	70·38	78·93	16·38	1·48	0·33	2·72	2·56	9·30	13·71	5·0	57·15	10·67
4. Sunflower green	24·04	53·84	44·57	48·75	20·22	71·60	78·89	11·28	1·44	0·32	1·02	1·79	6·72	8·51	4·8	34·12	7·17
5. *Bajra* green	21·64	63·50	58·66	66·67	59·74	61·78	68·75	13·94	1·21	0·22	4·12	0·93	7·27	10·29	12·9	47·64	4·31
6. Elephant grass green.	22·17	62·20	54·38	59·04	63·40	62·46	64·75	13·79	1·60	0·27	4·68	0·62	6·61	9·41	18·9	42·45	2·80
7. Guinea grass green.	25·10	56·46	41·20	41·88	57·74	59·29	59·76	14·17	1·26	0·16	5·27	0·78	6·70	9·27	15·3	36·95	3·11
8. Sudan grass green.	35·36	46·49	...	32·12	57·89	27·69	50·01	16·44	...	0·18	6·55	0·40	9·33	11·52	41·2	32·58	1·13
9. *Juar* green	32·98	56·33	19·58	38·61	61·03	36·64	61·27	18·58	0·57	0·19	7·01	0·41	10·42	12·90	43·7	39·12	1·24
10. *Juar* No. 3 green	39·75	56·77	17·97	41·67	60·15	43·10	61·60	22·57	0·70	0·28	6·50	0·84	13·91	16·95	25·4	42·64	2·11
11. *Juar* No. 11 green.	38·98	50·96	10·32	31·94	53·52	21·90	58·83	19·87	0·60	0·17	5·63	0·32	13·87	15·04	61·3	38·59	0·82
12. *Juar* No. 22 green.	38·60	54·43	...	12·92	57·32	...	64·52	21·01	...	0·06	6·46	...	15·50	16·94	...	43·89	...
13. Berseem hay	87·23	64·20	42·02	29·13	48·80	69·99	76·60	56·07	5·16	0·30	9·04	9·72	31·80	39·00	4·2	45·75	11·15
14. Lyallpur *Dub* grass hay.	91·27	44·50	16·30	27·25	53·83	54·42	46·41	40·62	1·87	0·34	8·92	5·51	23·97	29·10	6·1	31·87	6·04
15. Gram *bhusa*	90·60	41·78	33·61	...	40·10	40·15	47·25	37·86	4·06	...	16·15	2·18	15·29	10·23	14·5	11·29	2·41
16. Wheat *bhusa*	92·40	48·71	...	35·55	61·15	...	52·51	45·01	...	0·34	23·82	...	21·51	23·26	...	25·17	...

ESTIMATION OF VITAMIN-A RESERVE IN THE LIVERS OF SOME FARM ANIMALS

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INTRODUCTION

A considerable amount of discussion has centred round the suggestion of Green and Mellanby [1928] that vitamin-A increases the resistance of the animal body to bacterial infection, and the clinical work carried out by Mellanby and Green [1929] on the effect of vitamin-A therapy in cases of puerperal septicaemia, which gave apparently beneficial results, appeared to indicate that the administration of this vitamin might find a wide application in the treatment of infectious diseases. This hope has not so far been realised. Thus Harris, Innes and Griffith [1932] found that the infections observed in cases of vitamin-A deficiency are of a special type limited in origin to epithelial tissues, and not seen in the absence of neighbouring keratinization, and that the existing data afforded no basis for the belief that vitamin-A therapy was likely to be effective in combating acute general infections due to specific pathogenic micro-organisms, or in those clinical toxaemias and infectious diseases which are unassociated with the peculiar structural breakdown of epithelial tissue, and the attendant localised infection, which characterises the vitamin deficiency. The observations of Wolff [1932] and Moore [1932] on the vitamin-A reserve of human livers in health and disease have also shown that it is extremely difficult to correlate a low reserve of vitamin-A in liver with any particular type of infection. The trend of Moore's work, however, suggests that partial vitamin-A deficiency, or the state of multiple malnutrition which it must usually imply, may be of importance in the etiology of some types of infection under the conditions usually observed in clinical practice. It is also now realised that in many cases vitamin-A therapy may not increase the storage of this vitamin in the liver. Thus Green [1932] found that the vitamin-A reserves were low in most puerperal cases, and that they might remain low even after large amounts of vitamin-A had been administered therapeutically. He gives reasons for believing that this is not due to lack of absorption of vitamin-A when taken by the mouth, or to significant loss through urinary

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excretion, but rather to a failure on the part of the liver cells to assimilate and elaborate the vitamin when it reaches them in large quantity before it is either destroyed or passes into general circulation. As the livers of diabetics contained, according to Wolff and Moore, a very high reserve of vitamin-A due to the ingestion of the large quantity of carotene obtained from the vegetable diet, it appears that, if Green's explanation is considered correct, the activity of liver cells in storing vitamin-A may vary in different diseases. It was, therefore, felt desirable to obtain some data on the vitamin-A reserve in the livers of some farm animals, in health and in disease, and in this preliminary report the results obtained are given.

METHOD OF VITAMIN-A ASSAY

The Carr-Price method of estimating the vitamin, as modified by Davies [1933], has been used. Usually 5 grms. of liver was saponified with 10 c.c. of aqueous 5 to 10 per cent potassium hydroxide, and the unsaponified fraction twice extracted by means of ether, with the addition of some alcohol for the first extraction. The ether fraction was finally freed from water by shaking with anhydrous sodium sulphate and then quickly evaporated. In all essential particulars, the procedure adopted by Davies was used, and the method in our hands has given quite good results. The tintometer used was the B. D. H. pattern with artificial light attachment. The results are expressed in Moore's units per gram of the liver to the nearest significant figure. In all the data given in this paper, only the blue units are tabulated. The yellow or the red units of the tintometric readings have not been tabulated.

Having decided on the method of assay, it was thought necessary to determine (1) if there was any difference between the vitamin content of the central and the peripheral portion of the liver, (2) if decomposition for 4 or 5 days at room temperature would have any effect on the vitamin content, as it was realised that in some cases it was possible that the liver might not be sampled and put into potassium hydroxide solution for some days, and (3) if the relation between the tintometer blue units and the concentration of the unsaponifiable fraction actually used in the experiment (indirectly giving the amount of original liver) would be linear. In Tables I, II and III, these results are shown.

From the data given in Table I, it is apparent that there is a slight difference in the vitamin content of the central and the peripheral portion of the liver, the value of the central portion being higher and that decomposition for four or five days (without KOH) does not materially affect the vitamin value (Compare Davies, *loc cit.*). The results given in Tables II and III (excepting those of the goat) are shown graphically in Figs. I and II.

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FIG. I

HILL BULL

Amount of liver - milligrammes
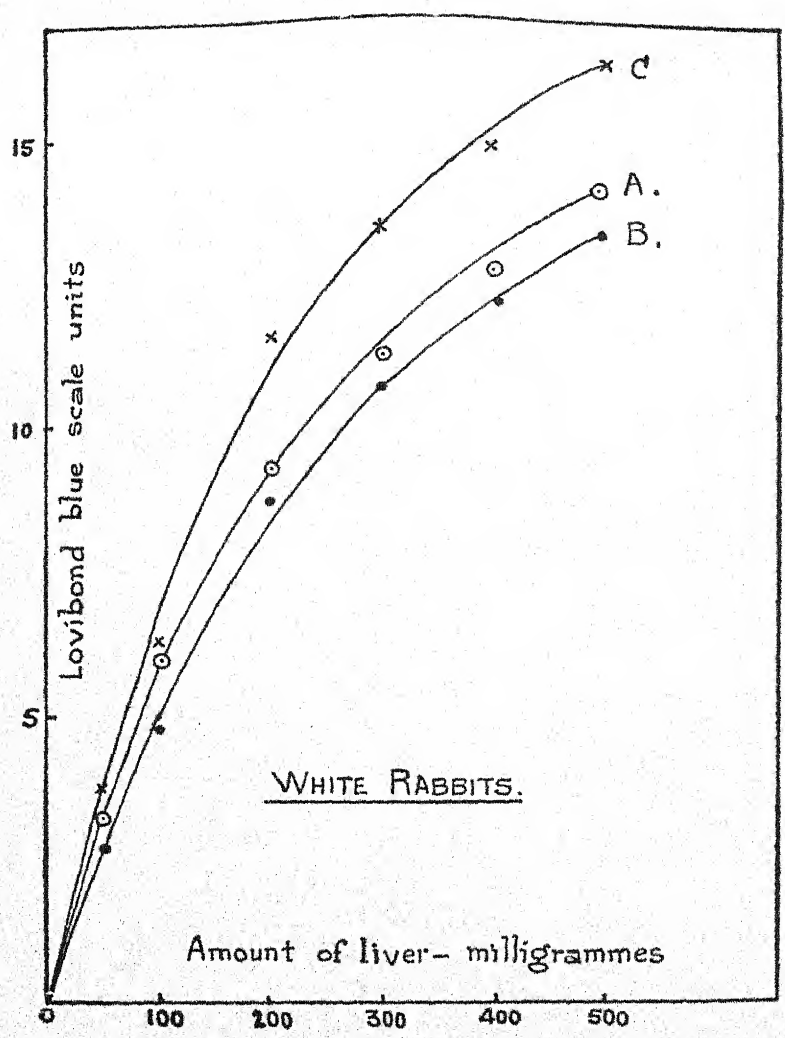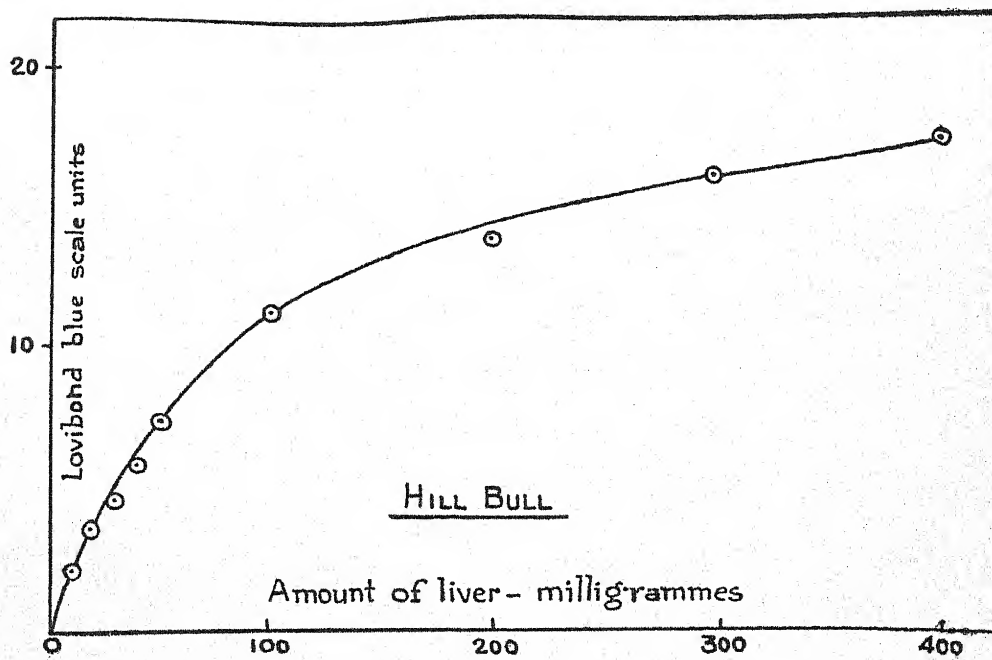
FIG. II

TABLE I

Nature of the sample	Weight of original liver represented by the chloroform solution of the unsaponifiable matter actually used for the tintometric estimation	Blue units per gram of liver (Moore)
Healthy Bull No. 15.		
(*a*) Central portion	} 20 mg.	400
(*b*) Peripheral portion		388
Healthy Bull No. 26.		
(*a*) Central portion	} 40 mg.	250
(*b*) Peripheral portion		200
Healthy Goat A.		
(*a*) Central portion		1,300
(*b*) Peripheral portion		1,175
(*c*) Central portion left at room temperature without KOH for 5 days.	} 10 mg.	1,325
(*d*) Central portion left in the refrigerator for 5 days as control.		1,275
Healthy Goat B.		
(*a*) Central portion		1,075
(*b*) Peripheral portion		1,000
(*c*) Central portion left at room temperature	} 10 mg.	1,100
(*d*) Central portion left in the refrigerator as control		1,075

TABLE II

Relation between concentration of the liver and the blue units

Weight in mg. of original liver	Rabbit A		Rabbit B		Rabbit C	
	Tintometric blue readings (Lovibond)	Blue units	Tintometric readings	Blue units	Tintometric readings	Blue units
50 . .	3·2	160	2·6	130	3·7	185
100 . .	6·0	150	4·8	120	6·2	155
200 . .	9·3	116	8·8	110	11·6	145
300 . .	11·3	95	10·8	90	13·5	113
400 . .	12·8	80	12·2	76	14·9	93
500 . .	14·2	71	13·3	66	16·3	82

TABLE III

Relation between concentration of the liver and the blue units

Specimen	Weight in mg. of original liver	Tintometric readings	Blue units
Healthy Bull	10	2·0	500
	20	3·5	438
	30	4·5	375
	40	5·8	362
	50	7·2	360
	100	11·0	275
	200	13·8	173
	300	16·0	133
	400	17·6	110
Goat affected with rinderpest . .	20	4·7	591
	40	7·2	450
	60	9·4	390
	80	11·2	350
	100	13·3	333

It will be observed that in none of these cases is the relation between the concentration of liver and the tintometric readings linear, although the unsaponifiable fraction has been used in the test. [Compare Norris and Church, 1930]. For obvious reasons, tests could not be carried out at very low dilutions. Our results are in general agreement with those available in the literature. [*Cf.* Chakravorty, Mukherjee and Guha, 1933 ; Datta and Banerjee, 1934, on fish oils]. From these

data it appeared that for comparative purposes, only the central portions of the livers should be saponified and that as far as possible, blue units obtained from the same amount of the original liver should be compared. It has, however, been found that the latter requirement is almost impossible in practice, because even among the same species, low amounts of the liver extract of healthy animals gave measurable Lovibond readings, whereas with the diseased ones, comparatively much greater amounts of the liver extract had to be used for accurate measurement of the colour. While, therefore, an attempt was made to use the same amount of the liver for the colour estimation, a second arbitrary standard of colour was also chosen, namely between 3 and 5 blue of the Lovibond scale (as suggested by Davies) and suitable amounts of material were used to give a blue colour with the antimony trichloride reagent within this range. Without these precautions, especially the weight of the material used, a comparison between the blue units of animal livers, even of the same species, loses much of its value because of the great variation in the Moore units per grm. of liver when different amounts of the liver are used for their determination. In Tables IV, V, VI and VII, the vitamin-A reserve in the livers of several species of animals under different conditions are shown. The actual amount of liver used in the determination of the blue units is shown in the tables in every case. It must be noted that an accurate comparison between two or several animals having a very high and very low vitamin content is not possible under the conditions found in this investigation, and as such only a rough comparison could be made.

TABLE IV

Hill Bulls

Serial No.	Brand No.	Condition prior to death or slaughter	Feed	Weight of liver used in mg.	Blue units
1	45	Healthy	Grain, green pasture, hay.	20	400
2	9	Do.	Do. .	20	438
3	26	Do.	Do. .	20	250
4	120	Do.	Ten days on a vitamin A deficient ration; no green grass.	20	250
5	144	Do.	Do. .	40	218
6	955	Experimental T. B. case. Four lymph glands and left lung affected.	Grain; green fodder, hay.	100	33

TABLE IV—contd.

Serial No.	Brand No.	Condition prior to death or slaughter	Feed	Weight of liver used in mgs.	Blue units
7	414	Experimental T. B. Injected with 10 mgm. bovine T. B. culture subcutaneously and later intravenously. Condition good. Killed after a year. No *post mortem* lesions.	Grain, green fodder, hay.	50	225
8	145	Experimental T. B. *Post mortem* lesions positive. Condition fair.	Do.	50	160
9	457	Recovered from Rinderpest. Condition rather poor ; died of acute gastro-enteritis.	Only hay during the disease.	100	130
10	22	Rinderpest : died on the 14th day : Coccidiosis as well. Condition poor.	Do.	100	80
11	1162	Rinderpest : died on the 14th day : Liver icteric.	Grain and hay	100	67
12	107	Rinderpest : died on the 12th day.	Hay	50	170
13	582	Recovered from rinderpest : healthy : Condition good.	Grain, hay and green fodder.	50	215
14	156	Rinderpest : destroyed on the 5th day. Condition fair.	Hay	50	165
15	636	Suffering from Theileriasis ; liver icteric ; Condition poor.	Grain, hay and green fodder.	100	78
16	55	Animal vaccinated against Blackquarter. Reacted and survived. Subsequently infected *per os* with *B. pyocaeneous* with negative result. Destroyed. *Post mortem.* Petecheal areas in liver ; fatty changes, mild congestion and early cirrhosis in the liver.	Do.	100	50

TABLE V

Hill goats

Serial No	Brand No.	Condition prior to death or slaughter	Feed	Weight of liver used in mgs.	Blue units
1	*Nil*	Healthy	Grain and green leaves.	20	1,125
2	,,	Do.	Do. .	20	913
3	,,	Do.	Do. .	10	1,150
4	,,	Do.	Do. .	20	725
5	,,	Do.	Do. .	20	800
6	..	Affected with Rinderpest virus ; destroyed on the 5th day : Condition fair.	Green leaves *ad. lib.*	200	75
7	55	Recovered from Rinderpest ; died of pneumonia and debility : Condition very poor.	Do. .	100	20
8	121	Affected with Rinderpest. Died on the 4th day.	Do. .	20	591
9	99	Rinderpest. Destroyed on the 5th day.	Do. .	20	388
10	122	Recovered from Rinderpest. Died of penumonia and debility after about 23 days of virus inoculation.	Grain and green leaves.	20	588
11	103	Discontinued from Rinderpest and Ecthyma. Died of pneumonia and debility.	Do. .	20	600
12	139	Rinderpest. Destroyed on the 5th day.	Green leaves .	50	165
13	137	Rinderpest. Died on the 6th day—peritonitis.	Do. .	20	375
14	130	Rinderpest. Destroyed on the 18th day.	Grain and green leaves.	20	500

TABLE VI

White Rabbits

Serial No.	Brand No.	Condition prior to death or slaughter	Feed	Weight of liver used in mgs.	Blue units
1	..	Healthy	Grain and green vegetables.	50	160
2	..	Do.	Do. .	50	130
3	..	Do.	Do. .	50	185

TABLE VII

Horses

Serial No.	Brand No.	Condition prior to death or slaughter	Feed	Weight of liver used in mgs.	Blue units
1	364	Suffered from chronic paraplegia. Condition poor; destroyed.	Green fodder, grain and hay.	200	88
2	..	Animal suffered from "Kumri"; destroyed.	Do. .	200	53
3	386	Foal; 2 months old. Sprayed intranasally with emulsion of *C. equi*. Died of pneumonia.	Milk .	300	50

TABLE VIII

Dairy calf, Holstein Hariana : No. 269 : 2 months old : suffered from diarrhœa and died. *Post mortem* observation—Congestion of the intestine. Blue units on 200 mgs. of liver—41.

DISCUSSION

In considering the foregoing results, it has to be remembered that the data are not sufficiently numerous to allow any statistical analysis, and any conclusion which may be drawn is of a tentative nature. It may be recalled here that Wolff studied more than 900 cases and Moore studied over 300 cases. In one

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respect, however, the data obtained on bulls and goats may be considered to be more reliable than those obtainable on human livers, because in the case of these animals, the conditions are better defined, the food supply can be standardised and the history of the individual animals is known. In this preliminary work, however, we have not been able to standardise the feed and chose animals with comparable nutritional history. It may be that for this reason the vitamin-A reserve in the livers of even healthy animals has shown considerable variation. Thus the values of vitamin reserve in healthy hill bulls lie within the range of 250 to 438 blue units in three animals (a very insufficient number ; numbers 7 and 13 might be considered practically healthy) with an average of 362 units, and in the case of healthy goats, within a range of 725 to 1,125 in five animals with an average of 943 units. In the case of hill bulls, 10 days on a vitamin-A deficient diet appeared to lower the reserve of this vitamin in the liver only to a slight degree. It is, therefore, of interest to note that hardly in any case do we find a blue value in the liver of an animal (bull or goat) suffering from a disease closely approaching the average blue value observed in the livers of healthy animals of the same species. This observation seems to indicate that in certain diseases such as rinderpest in bulls and goats, there is a possibility that the vitamin-A reserve in the livers may be depleted. Whether this is a direct effect of the disease or due to inanition following upon the inappetence and digestive irregularities due to the lesions caused by the disease cannot at present be stated. It may be pointed out here that in our routine rinderpest work, the animals (bulls) actually going through the disease are not supplied with any green pasture (maximum period from the onset, 15 days). In the case of goats, green leaves are supplied *ad libitum*, but no grain ration is given during this infection. As already observed in the case of bulls on vitamin-A deficient ration, this withholding of green fodder for a short period is not likely by itself to lower to any considerable degree the vitamin reserve in the livers of bulls going through an attack of rinderpest. Advanced tuberculosis and Theileriasis in bulls also apparently lead to a diminution of the vitamin reserve. One animal, Bull 55, was drenched with B. Pyocaeneous, but did not become clinically infected. Curiously enough, this animal's liver also gave a low vitamin-A value. A histopathological examination of the liver showed mild congestion and early cirrhosis.

In the case of rabbits only healthy animal livers have been examined to provide comparative data. It is well-known from Mellanby's work [1934] that avitaminosis A produces serious disorders in this species of animal.

In the case of horses, apart from the foal which might be expected to have a low vitamin reserve, both the other animals had suffered from paralysis and were destroyed. As far as is known, the diet supplied was of the usual type and contained green fodder. The blue values appear to be low, but as no livers from healthy animals were available, no comparison can be made.

The vitamin reserve of the calf liver seems also to be low.

No other animals have as yet been studied by us, but some references, often of a qualitative nature, are available in the literature. Thus Rosenheim and Webster [1927] stated that fats of the sheep, calf and ox contain on the average as much as ten times the amount of vitamin-A as a good New Foundland cod liver oil. Wilson [1927] made a comparative study of the vitamin-A content of extracts of the livers of several animals. Wolff, Overhoff and van Eckelen [1930] studied rabbit liver and Dann [1932] gives some data showing the passage of vitamin-A from the mother to the foetus in the rabbit. Moore [1932] has given some quantitative data on the vitamin-A content of the livers of different breeds of cattle and has shown that breed has a very important effect in this connection and Davies has given some data on sheep, ox and pig livers. Some work on the vitamin-A content of pig liver has been reported by Dunlop [1934, 1935].

Lastly, mention must be made of the interesting investigation of Guilbert and Hart [1934] on the storage of vitamin-A in cattle and of Guilbert and Hinshaw [1934] on vitamin-A in poultry livers.

If a comparative study of the storage capacity of the livers of healthy animals of different species for vitamin-A as given in this paper is made, it is found that goat liver has a reserve which is more than twice that of the bull liver and six times that of rabbit liver. Since carotene, which is the main source of vitamin-A in these animals, is supplied in fairly high amounts through the green feed, it is likely that the marked variation in the vitamin-A reserve of the livers in these three species of animals is due to a difference in the metabolism of carotene by these species. Whatever the explanation may be, goat liver appears to be a good source of vitamin-A.

SUMMARY

A study has been made of the vitamin-A reserve in livers of several species of animals by the antimony trichloride test. It is found that the central portion of the livers usually contains more vitamin than the peripheral portion, that reasonable delay in sampling the liver does not produce any noticeable change in the vitamin-A content and that the relation between the blue units and the amount of the material used for estimation is not linear at the dilutions studied in this investigation. There is a possibility that certain diseases, e.g., rinderpest in bulls and goats, and advanced tuberculosis and Theileriasis in bulls may lower the vitamin-A reserve, but this requires to be confirmed. Healthy goat liver contains a much higher amount of vitamin-A than that of bulls, rabbits and (probably) horses, and appears to be a good source of this vitamin.

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AN AROMA-PRODUCING LACTIC ACID ORGANISM ISOLATED FROM INDIAN DAIRY PRODUCTS

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Empirical natural starters in the form of curds or buttermilk for making butter continue to be used in the Indian homes and by *gowallas*, the professional dairy men in India, even up to the present time. Such starters were also in common usage in Europe and America till about the year 1890, when after the rise of the science of bacteriology, a discovery was made independently by Storch in Denmark, Weigman in Germany and Conn in United States of America that certain bacteria, which produce lactic acid from lactose in milk, were the causative agents in the souring of milk or the ripening of cream. The investigations of Storch led the way to the use of bacterial cultures first in Denmark for ripening cream in place of the empirical starters consisting of buttermilk from previous churnings of good butter which were used before. The chief findings of the classical discovery in the process of cream ripening and the investigations immediately following it show that different kinds of lactic acid bacteria induce different kinds of souring : (1) some give a clean sour taste, (2) some produce high acidity, (3) others give an unpleasant tang, (4) while still others give a mildly acid but strongly aromatic taste.

The bacterial cultures used as starters were, at first, considered to be pure cultures of a single variety of the lactic acid organisms. The organism commonly found in such culture starters is the variety which is predominant in sour milks and recognised as *Streptococcus lactis*, (Lister) Lohnis. Orla Jensen has named the variety occurring in well-ripened cream in dairies as *Streptococcus cremoris* (Orla Jensen) and distinguishes it as different from *S. lactis* on account of some differences in morphological and cultural characters. Both these organisms, however, produce the same amount of lactic acid from lactose in milk and are similar in many respects. It was soon realised that pasteurised cream, when inoculated with pure cultures of any of these lactic acid organisms and ripened, did not always yield a butter of full aromatic odour and mild acid flavour as was obtained with some natural starters, *i.e.*, mixed cultures used in certain dairies. This led to the suggestion that there may be some other organisms responsible for the flavour in butter from particular dairies which were renowned for their butter. A search was, therefore, continued by bacteriologists for isolating and securing cultures of

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bacteria, other tann the lactic acid organism, in the dairy starters which produced butter with full aroma and good flavour, as well as in commercial starters made from such dairy starters. The discovery that two different types of bacteria are present in good starters was made in the year 1919 independently in three countries. Bokehout and De Vries [1919] in Holland, Hammer and Bailey [1919] in U. S. A., and Storch [1919] in Denmark found that mixed cultures of *S. lactis* (Lister) Lohnis or *S. cremoris* (Orla Jensen) and another type of organism when inoculated in pasteurised cream produce the characteristic aroma of good butter. This type of organism is called *S. citrovorus* or *S. paracitrovorus* by Hammer, ' X ' bacteria by Storch and ' *Betacoccus cremoris* ' by Orla Jensen. Although named differently, these organisms appear to have the common characteristic of producing aroma in butter.

While engaged in an investigation of lactic acid organisms in *dahi*, the principal starter used by Indians, we made attempts to find out whether the dairy products in India contained any flavour-forming organisms, similar to those just mentioned and whether their activity continues throughout the year or is restricted to any particular season. In this connection we examined several samples of milk, *dahi* and butter by plating on purple lactose agar and making pure cultures from single colonies appearing on the plates. While examining the cultural characteristics of the several organisms isolated by us we came across one organism which coagulated milk at 21°C. without any gas and with little acidity after incubation for 24 hrs. The flavour of the curd produced by this organism was different from the flavour produced by the usual lactic acid organisms present in *dahi*. Hence this organism appeared to combine in it the characteristics of both the lactic acid producing and aroma-producing organisms, and we considered it likely to be useful in dairy practice. A detailed study of the cultural and morphological characteristics of pure cultures of this organism was, therefore, made and its ability to produce flavour in butter was tested by using it as a starter for ripening pasteurised cream. The results of this study are included in this paper.

EXPERIMENTAL

(1) *Isolation* :—Several tubes of sterile milk were inoculated with a small quantity of each of the following :

(*a*) Butter of various brands,

(*b*) Spontaneously soured creams,

(*c*) *Dahi* from different localities,

(*d*) Milk incubated for a few hours after addition of lime juice. The inoculated tubes were incubated at 21°C. for four days. Out of these tubes those which showed smooth coagulation were after necessary dilution plated on purple lactose agar, and the plates incubated at 21°C.

From samples of (*a*), (*b*) and (*d*) several strains of streptococci were obtained of which many proved to be *S. lactis* (Lister) Lohnis, while a few isolated from butter samples appeared different. From samples of (*c*), in addition to streptococci, another organism similar to the *Bulgaricus* type was most frequently isolated.

From milk samples in the cold season, strains of streptococci, similar to those from butter samples, could be only occasionally isolated.

(2) *The ability of the different organisms to produce volatile acidity.*—Since lactic acid is odourless, previous investigators had considered the mild acid flavour in samples of good butter to be due to the formation of some volatile acid produced during ripening of the cream by the organisms in starters. The fact that good starters, when inoculated in milk and incubated for a week and then distilled—after acidifying by sulphuric acid—with steam, gave a large amount of volatile acid, whilst indifferent starters gave a decidedly lesser amount, appeared to lend support to the above view. It was further found that the addition of citric acid to milk or cream considerably increased the volatile acid produced by the organisms in good starters.

The ability of some of the organisms to produce volatile acid in milk with and without the addition of citric acid was, therefore, tested several times to find out whether they possess this quality of good starters. 250 c.c. of milk was used in each flask for inoculation with the respective organisms and after a week's period of incubation, the whole quantity of milk was distilled with the addition of 15 c.c. N H_2SO_4 in a current of steam, and the distillate collected until it amounted to 1,000 c.c. and titrated with $N/10$. NaOH using phenolphthalein as indicator. The results of one typical experiment are given in the following table :—

TABLE I

Volatile acidity produced by different organisms

Origin of the organism	No. of c.c. of $N/10$. NaOH required for neutralization of distillate (Average of 4 tubes)	
	Milk	Milk and citric acid (0·40%)
1. *S. lactis* from soured cream	8·0	10·0
2. Bulgaricus type from *dahi*	12·0	14·5
3. Streptococcus from a sample of good butter	24·7	43·9
4. Streptococcus from milk and lemon juice	9·5	10·5

Three of the organisms did not produce any appreciable amount of volatile acid nor did they show any marked increase in volatile acidity after addition of citric acid, but the streptococcus from butter shows in milk alone a high volatile acidity, which is further increased by the addition of citric acid to milk. The number of c.cs. of $N/10$ NaOH required for neutralizing the distillate from S. citrovorus culture as recorded by Hammer [1920] are 20·3 without citric acid and 57·3 with citric acid on an average, while for S. paracitrovorus the numbers recorded by Hammer [1923] are 28·6 in milk without citric acid and 73·9 in milk with citric acid, the least number of c.c. of $N/10$ NaOH required for neutralising the distillate from milk and citric acid culture of S. paracitrovorus being 44·4. Judged by a comparison with these two sets of results, the streptococcus isolated by us from butter appears to be a citric acid fermenter more akin to S. citrovorus.

(3) *Relation of temperature to growth and activity of the streptococcus from butter.*—10 c.c. tubes of sterile separated milk were inoculated with the organism and incubated at 21°C., 30°C. and 37°C. and the acidity determined by titration with $N/10$ NaOH after 24 hours and 72 hours. The following table gives the number of c.c. of $N/10$ NaOH required to neutralise the acid formed ; the figures represent the average for four tubes.

TABLE II

Temperature of incubation	After 24 hrs.	After 72 hrs.
21° C	8·1	10·4
30° C	9·4	11·4
37° C	10·2	10·1

The optimum temperature for the growth and formation of lactic acid by this streptococcus from the above table is regarded to be 30°C. because it has given the highest acidity at this temperature after 72 hours, although the organism shows nearly equal amount of acid at the other temperatures.

(4) *Cream ripening tests.*—Cream ripening tests with different organisms were next carried out to see which of them gives aroma to the butter. Three lots of cream, each 4 ounces, were heated and held at 100°C. for half an hour and separately inoculated after cooling with the following organisms :—

(i) *Streptococcus lactis.*

(ii) *Bulgaricus* type from *dahi.*

(iii) *Streptococcus* from butter.

After keeping for 48 hours at 21°C. the creams were churned and the butter separated out from each. The samples of butter were submitted to different

persons for judging the taste and flavour. As a combined result of the judgments of several persons, it was found that :

    (*i*) *Streptococcus lactis* butter had no flavour and was mildly acidic. This butter stood second in the order of merit according to the judgment by taste of different persons.

    (*ii*) *Bulgaricus* type butter had a decided acid flavour and a strong acid taste. This butter stood third in the order of merit.

    (*iii*) *Streptococcus* from good butter gave a mildly acid tasting butter. It had also aroma and flavour. This butter stood first in the order of merit.

The experiment was repeated three times with identical results.

(5) *Large-scale cream-ripening tests.*—A large-scale experiment on the production of butter from cream was conducted at the Institute of Dairying and Animal Husbandry at Bangalore in June 1933. The cream used was mixed (morning and evening). The morning cream, after separation of milk which had been pasteurised, was kept without any further treatment till the time of inoculation. No effort was made to prevent any natural souring of the morning cream except that it was kept immediately after separation of the pasteurised milk in a cold temperature room. After separation of the evening cream, both were mixed and divided into lots of 9 lbs. each in separate buckets. The starters for use were prepared in separated milk from pure cultures. The cream was ripened for 24 hours at the dairy room temperature, about 22°C., to 25°C., and then transferred to the cold room. The butters were scored according to the combined judgment of several persons including the Imperial Dairy Expert, the members of the staff and some students of the Dairy Institute with the request that they should place the butters in order of merit ; and the final places were determined by adding the numbers of the order which each butter obtained. The butter with the smallest number was considered to have obtained the first place. As this common general judgment tallied with the expert judgment of the Imperial Dairy Expert, no attempt was made to have any more accurate scoring of the butters. The results are given in the following Table :—

TABLE III

Organism tested	Per cent acidity of ripened cream	The place obtained by the butter from organisms in order of merit
Dahi organism (resembling the *Bulgaricus* type of lactic acid organisms).	0·34	2
Streptococcus lactis	0·43	3
Culture of the organism (*Streptococcus*) described in this paper	0·35	1
The local starter used in the Bangalore Dairy . .	0·37	4

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As this result confirmed the one obtained at Pusa, there is little doubt of the superiority of this organism as a starter in dairy practice in India for producing flavour in butter. It may not be out of place to point out here that the superiority claimed is in respect of the use of single cultures only. It is possible and perhaps natural to find, as was our experience in other investigations not reported here, that a mixture of two different cultures may give a better-flavoured butter than the one produced by a pure culture of the streptococcus under study used singly. The cultures of the flavour-producing streptococcus investigated by us may not be necessary for those dairies which may already be using starters which contain the flavour-producing organisms in addition to the usual lactic acid organisms ; but dairies which are using indifferent starters will certainly benefit by resorting to the use of cultures of the flavour-producing organisms similar to the one investigated by us.

(6) *Test for aroma and flavour-producing substances by the organisms in cultures.*—It was at first generally believed that the volatile acids produced by the organisms were the chief cause of aroma and flavour in butter, because Evans *et al* [1914], Hart *et al* [1914] and Evans [1918] had isolated and studied organisms producing volatile acids from *cheddar* cheese and suggested that they are responsible for volatile fatty acids and the flavour found to be developed during the ripening of cheese ; and Hammer and Bailey [1919] had found volatile acids produced by the citric acid fermenters in amounts equal to that of good starters. Recent studies carried out in Europe, by Schmalfuss [1928], Schmalfuss and Barthmeyer [1928] and Van Niel, Kluyver and Derx [1929] have, however, clearly shown that it is the presence of an organic compound " diacetyl " having the formula $CH_3 \cdot CO \cdot CO \cdot CH_3$,—the oxidation product of acetyl methyl carbinol—which is responsible for the development of aroma in butter although the volatile acids as a factor in the development of flavour is not ruled out. Michaelin, Farmer and Hammer [1933] conducted a series of experiments with citric acid fermenters associated with *S. lactis* in milk cultures and have confirmed the above results obtained in Europe. These investigators found that the organisms *S. citrovorus* and *S. paracitrovorus* produce considerable amounts of the above-named substances in milk to which citric acid is added and likewise impart satisfactory flavour and aroma to butter when inoculated conjointly with *S. lactis*. We, therefore, attempted to find out whether " acetyl-methyl-carbinol and diacetyl " are formed in milk alone and in milk to which citric acid has been added by the organism isolated by us. For comparison we used two lactic acid organisms isolated from *dahi* and also one of the several cultures which were kindly supplied to us by Dr. Hammer of the Dairy Section of the Iowa State College of Agriculture. The procedure of determination of acetyl-methyl-carbinol-+-diacetyl was the same as outlined by Michaelin *et al* [1933] and consisted in steam-distilling 200 grms. portions of milk cultures to which 40 c.c. of ferric chloride solution had been added and

collecting the distillate in fractions. To the first fraction (25 c.c.) of the distillate were added a mixture of the following solutions:

2 c.c. of hydroxylamine hydrochloride (20 per cent solution).

3-5 c.c. of sodium acetate (20 per cent solution), and

1-2 c.c. of nickel chloride (10 per cent solution) and warmed to 80°c.

If the first fraction showed a significant precipitate, the reagents were added to the second fraction (75 c.c) of the distillate. The quantities of the reagents given are sufficient for about hundred mgms. diacetyl according to Van Niel [1927]. The distillate with the reagents was allowed to stand for 24 hours for complete crystallisation. This can also be accomplished by heating it to one hour on a water-bath at 80°C. The nickel salt precipitated is then filtered into a weighed crucible, washed and dried to a constant weight at 105°C.—110°C. and the results recorded as mgms. of nickel salt equivalent to acetyl-methyl-carbinol+ diacetyl per 200 grms. of milk. The quantity of ferric chloride solution used to oxidise acetyl-methyl-carbinol to diacetyl was first determined by trial distillations of milk to which were added watery solutions of these substances which were kindly supplied as samples by a firm of manufacturing chemists in Holland. The results of the experiment are given in the following table.

TABLE IV

Mgms. of nickel salt equivalent to acetyl-methyl-carbinol and diacetyl

Culture used	No. of days of Incubation	Milk alone	Milk plus 0·5 per cent citric acid
Streptococcus under study	4	Traces	30
Streptococcus under study+Hammer's culture M. 29.	4	Traces	42
Streptococcus lactis	5	*Nil*	Traces
Streptococcus under study	5	Traces	77
Streptococcus under study+Hammer's culture, M. 29.	5	Traces	57
Hammer's culture, M. 29	5	Traces	49
Streptococcus under study	7	*Nil*	16

From the Table IV, it is clear that in milk they do not produce any detectable quantities of acetyl-methyl-carbinol+diacetyl, but with the addition of citric acid to milk, both M. 29 and the organism under investigation produce a considerable amount of acetyl-methyl-carbinol+diacetyl, while *S. lactis* does not produce any, even with citric acid addition to milk. Further, we find that after seven days' incubation, the amount is much less than that after five days of incubation with the organism under study. This appears to show that the organism first

forms acetyl-methyl-carbinol-and diacetyl, but afterwards utilises these compounds for its own use or decomposes them.   This would be in accordance with the results of Michaelin *et al* [1933].   A fresh experiment was, therefore, carried out and the products determined in separate flasks containing milk and citric acid which were inoculated with the organism under study and distilled with steam at different periods.   The results are given in the table below :—

<div align="center">TABLE V</div>

Mgms. of nickel salt equivalent to acetyl-methyl-carbinol and diacetyl in 200 grm. culture

Culture	No. of days	Milk+0·5 per cent citric acid
		Mgms.
Streptococcus under study	1	*Nil*
Ditto	2	7
Ditto	3	2S
Ditto	5	70
Ditto	7	18

These results show that the acetyl-methyl-carbinol+diacetyl is produced in small quantities in the first 48 hours of the culture and that it reaches its maximum on the fifth day and subsequently declines considerably on the seventh, rather suddenly, suggesting that it is lost not so much by evaporation as by being assimilated and decomposed by the organism itself for its own use.   Incidentally, this behaviour of the organism provides a useful hint that the time to be allowed to develop the aroma in the ripening of cream cannot be extended beyond a certain limit which can be ascertained with the cultures of starters used in dairies, by the above method.

(7) *Morphological and biochemical characters.*—We next turned our attention to a study of the morphological and bio-chemical characters of the organism. A description of these prepared from the results of our observations is as follows :—

Form.—Spherical or slightly longer than broad. Usually arranged in pairs in milk culture. Chain formation occurred in agar.

Size.—0·6-1·0 μ ×0·7-1·2 μ.

Motility.—The organisms have not shown motility at any time.

Staining reactions.—Stain readily with aniline stains ; gram positive.

Cultural characteristics :—

Whey agar streak.—After 24 hours at 21°C. small non-viscid closely grouped colonies precipitate in condensation water. Growth was feeble on Lemco agar.

Agar stab.—Slight growth at the surface, but more along the line of stab. Facultative anaerobic.

Purple lactose agar.—Medium changed to yellow after 24 hours' growth at 21°C. Under low magnification, circular rough-edged, very minute colonies which did not increase in size after further incubation.

Whey gelatine stab.—Gelatine not liquefied, growth along stab 24 hours, filiform ; little surface growth.

Bouillons.—No growth in plain bouillon. Sediment was observed in bouillons to which fructose, galactose, glucose, lactose and maltose had been added, but no growth in bouillons with glycerine, sucrose, mannitol or raffinose.

Potato growth was not evident.

Plain milk coagulates smoothly in 24 hours at 21°C. No gas production. Acidity was distinct.

Litmus milk.—Litmus was reduced and decolourised at first, but turned red afterwards and milk coagulated.

Biochemical features—

   Gas production.—No gas produced from any sugars or other fermentable materials.

   Acid production.—Distinct acid production was observed. 10 c.c. milk titrating on an average to 10·0 c.c. $N/10$ NaOH.

   Oxygen relation.—Facultative anaerobic.

Examination of these characteristics shows that the organism is closely related to *Streptococcus citrovorus* (Hammer) from which it differs in the fact that while *S. citrovorus* does not produce much acid and does not coagulate milk, this organism as described above produces acid and coagulates milk within 24 hours at 21°C. This organism differs also from *S. paracitrovorus* in producing stronger total acidity—about 0·9 per cent as compared with 0·4 per cent to 0·7 per cent total acidity produced by *S. paracitrovorus* and lesser amount of volatile acidity than *S. paracitrovorus*, as has been already observed. Thus, although a citric acid fermenter itself, the organism described by us differs from the two described by Hammer in its marked ability to produce a large amount of lactic acid in milk, we, therefore, propose to call it " *Streptococcus lactis aromaticus* " Nov. Sp., as it combines the property of producing lactic acid with the production of diacetyl or its precursor acetyl-methyl-carbinol in high amount in milk with citric acid.

Finally, we may be allowed to say that our aim in writing this paper is not so much to claim superiority for the organism described by us, but rather to draw attention to—

   (1) the rarity of the aroma-producing organisms in Indian butters,
   (2) the necessity of having the starters used in Indian dairies examined for the presence of aroma-producing organisms,

(3) the necessity of introducing such organisms where they may not be already present in the starters if improvement in aroma and flavour of the butter is desired, and

(4) the fact that we have a culture of such an organism which has been found useful in improving the aroma and flavour in comparison with the organisms usually present in many localities in India and with which a beginning may be made for improving the flavour of butter till such time as the dairies can develop their own starters containing the best aroma producers suited to their locality.

SUMMARY

Examination of a large number of samples of milk, cream and butter in India shows that the organisms producing aroma in butter are comparatively of rare occurrence in these products. Hence there is a necessity of introducing these in starters in dairies where they may not be present if the quality of the butter is to be improved. One such organism isolated by us from a sample of good butter and its ability to ripen cream properly have been studied, and these tests have shown that when used as a starter, a butter of better flavour and aroma is obtained as compared with the lactic acid organisms likely to be present under local conditions in several Indian dairies.

The cultural and morphological characters show the organism described by us to be similar to *S. citrovorus* and *S. paracitrovorus*, but on account of its marked ability to produce lactic acid, combined with the property of producing aroma in butter, we have named it *S. lactis aromaticus* nov. sp.

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IS *PIROPLASMA TAYLORI* SARWAR A VALID NEW SPECIES ?

BY

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I have read with interest the paper by S. M. Sarwar in the June (1935) Number of this *Journal*. He has briefly reviewed the previous literature dealing with records of various Piroplasmidea from goats and sheep both in this country and elsewhere, and given a fairly adequate description of a form that he came across in smears made in the course of a *post mortem* examination of a goat. There is a vast amount of literature dealing with Piroplasmidea, and good deal of confusion prevails as regards the nomenclature of various genera and species. It should, therefore, be the desire of every worker to avoid adding to that confusion, and before one describes any organism as a new species, he should see that his observations are correctly made and interpreted, and carefully compared with the existing descriptions of similar organisms. I have no fault to find with his description or the microphotographs and the coloured plates that accompany it, but on account of an error of interpretation he has been led to regard it as new species, and to place it in a genus to which it does not belong.

GENERAL REMARKS

Before dealing with the specific organism, I would like to make a few general remarks. The earliest record of piroplasms of goats in India is not of Krishna Iyer [1932], but of Lingard and Jennings [1904], who studied and figured the piroplasms from a large number of domestic animals including the goat. Christophers is quoted by Dschunkovsky and Urodschevich as having remarked that the parasites found in sheep in North and South India differ from each other and from the forms described from other countries. Towards the conclusion of his paper Mr. Sarwar observes as follows :

" The generic name of Piroplasma has been retained on account of the inadmissibility of the three recognised genera *Babesia, Nuttalia,* and *Theileria* ". I am aware of the fact that certain authorities stick to *Piroplasma* Patton [1895], as a generic name, instead of *Babesia* Starcovici [1893], which has priority over it ; and that *Nuttalia* Franca [1910], is now generally regarded as one of the synonyms of *Babesia* and not a separate genus. But I wonder what is the author's authority for considering *Theileria* Bettencourt, Franca and Borges [1907], as inadmissible : *Theileria* is a well established genus, which is so distinctly marked off from *Babesia*, that the two are placed in separate families by du Toit [1918],

(151)

Wenyon [1926], Reichenow [1929], and other authorities on the subject. Du Toit classified the Piroplasmidea into two families, *Babesidae* and *Theileridae*, each containing a number of genera. Wenyon considers the division into two families as justified, but regards each family as containing a single genus, *viz. Babesia* and *Theileria*. The characters of the families may be summed up as follows:

Family BABESIDAE Poche, 1913. Non-pigmented parasites of the red blood-corpuscles of mammals which multiply in the corpuscle by division into two or four. They are of varying size and shape, but usually arrange themselves in pairs of pear-shaped individuals. The forms in the corpuscles are asexually reproducing individuals, and possibly some are gametocytes. Genus *Babesia* Starcovici (1893).

Family THEILERIDAE du Toit, 1918. Non-pigmented parasites of the red blood corpuscles of mammals. Schizogony takes place in the endothelial cells of the capillaries of the internal organs and the schizonts ("Koch's blue bodies") produce a number of merozoites. The parasites finally invade the red corpuscles, within which they occur as round, ovoid, rod-like or irregular forms. They show no tendency towards a paired arrangement. The forms in the red corpuscles do not reproduce, and are possibly gametocytes. Genus *Theileria* Bettencourt, Franca, and Borges [1907].

It is by no means easy to decide whether a particular form met with in the peripheral blood is a *Babesia* or a *Theileria*. In the former, the organisms are pear-shaped, and reproduce in the blood-corpuscles into two or four daughter individuals which consequently show a tendency to pair. Sometimes, however, as many as a dozen or more parasites can occur together in a single corpuscle. This increase in numbers is due to budding or to multiple infection, and is not comparable to the multiple fission as it takes place in *Plasmodium*. The parasites produce no pigment, but destroy the corpuscles in which they are contained, and set free the haemoglobin which is excreted by the kidneys of the host, producing haemoglobinuria. In *Theileria*, on the other hand, schizogony takes place in the endothelial cells of the capillaries of the internal organs and forms produced there enter the red corpuscles and are seen in the peripheral blood. Unlike *Babesia*, they do not multiply in the red corpuscles. The blood is consequently not infective when inoculated to healthy animals unless endothelial cells containing schizonts happen to be present. Yakimov [1931] has given a useful and practical scheme of dividing the genus *Piroplasma* Patton into two sub-genera, *Piroplasma s. str.* and *Babesiella* Mesnil, dividing the species belonging to the latter into two groups again. This scheme should however be followed, if at all, if the species under consideration is definitely ascertained to belong to *Babesia* (*Piroplasma*) and not to *Theileria*.

SPECIFIC IDENTITY OF THE FORM

Now we shall turn our attention to the particular species under reference. According to the description of *Piroplasma taylori* as given by the author, the parasites are mostly ovoid or round, pear-shaped forms being rare. When single

parasites are found in a cell they measure 2 μ by 1·5 μ. Although single parasites are frequently seen, it is also not uncommon to find two, four, eight and sixteen elements in one red cell. Another feature is the occurrence of a fairly large number of parasites in extra-cellular forms. The occurrence of eight and sixteen elements in a red cell as also the occurrence in fairly large numbers of extra-cellular forms is quite unusual for a *Babesia*, and shows that the organism is a *Theileria*. But the author rules it out by his remark that " the absence of Koch's blue bodies in the smears excludes the possibility of this parasite belonging to the genus *Theileria* ". Now what are the extra-cellular bodies showing multiple division (*vide* his plates VI a and VII) *if they are not schizonts or Koch's blue bodies ? No doubt ordinarily in *Theileria* Koch's blue bodies are found in endothelial cells of internal organs, but they have also been observed in peripheral blood in certain species.

The author has compared his form with the three species of *Babesia* and with *Theileria hirca* previously known from goats. Although he mentions Dschunkovsky and Urodschevich's paper [1924] in his list of References, it is obvious that he did not consult the paper in the original, as the following misstatements of facts would clearly show. He mentions (p. 172)* that *T. hirci* is characterised by the presence of Koch's blue bodies in the lymphatic glands and internal organs, and in his table (p. 175)* states that the species is characterised by the presence of haemoglobinuria. In both these respects, the statements made are quite contrary to the original description. I quote the relevant portions below :—

" Elements similar to Koch's blue bodies or " Plasmakugeln " were also encountered in peripheral blood films. These bodies were round or oval, at times as large or larger than a blood corpuscle, and contained chromatin granules of varying size and shape. We were unable to examine the goats ' internal organs with a view to studying the parasites therein, this being unfortunate in view of the ' blue bodies ' above referred to ". Further on, under symptoms they state " Urine cloudy but no haemoglobinuria ".

I will further quote Dschunkovsky and Urodschevich's description of the form as seen in the peripheral blood, " the parasites occurring singly, in twos, rarely in threes, in the centre of the corpuscles. The parasites were mostly small and distinctly ring-shaped, others being bacillary or nail-like, oval or piriform " .... " Many extra-cellular parasites were observed, all were rounded. Rod or nail-like forms are rare. Cross-like forms of parasites occur, each member being piriform ".

A perusal of the above extracts will show that there is a complete resemblance between *Piroplasma taylori* Sarwar and *Theileria hirci* Dsch. and Urod., as regards form of the parasite, presence of Koch's blue bodies in the peripheral blood, and in the absence of haemoglobinuria, and the two must be regarded as identical.

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* *The Indian Journal of Vety. Science and Anim. Hus., Vol. V, Pt. II.*

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Theileria hirci Dsch. and Urod., was described from goats in Siberia in 1924. The same year Lestoquard recorded *Theileria ocis* as a parasite of sheep in Algeria, and found the schizonts in the liver, spleen, kidneys, and bone marrow, and observed a transient haemoglobinuria on the second day of the disease. Wenyon [1926] regards this species as identical with *T. hirci* Dsch. and Urod.

Other forms correctly referable to *Babesia* and *Plasmodium* have also been recorded from goats, but I shall not discuss them here.

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PLATE IV.
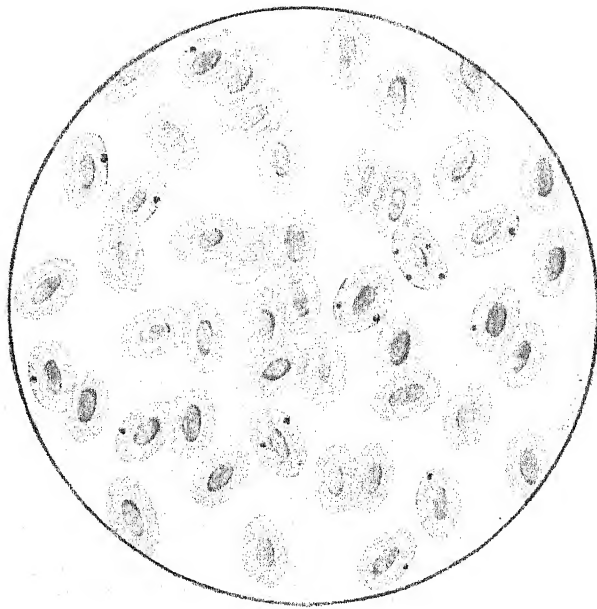
Hoemoproteus (*rileyi*) n. sp. in Peacock's blood.
(The drawing is not to scale)

HOEMOPROTEUS RILEYI (SP. NOV.) CAUSING A FATAL DISEASE IN INDIAN PEACOCK

BY

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(WITH PLATE IV.)

Several distinct species of pigmented parasites of the red blood corpuscles belonging to the genera *Hoemoproteus* and *Plasmodium* have been recorded from different species of birds. Although practically nothing in the case of members belonging to the genus *Hoemoproteus*, and very little in the case of members of the genus *Plasmodium*, is definitely known of the possibility of one and the same species occurring in different hosts, recent investigations have tended to prove that probably many of the several distinct species, primarily created only on the assumption that each parasite is specific to the host in which it occurs, do actually exist. Reference to the check list given by Wenyon [1926] and the available literature shows that pigmented parasites of the red blood corpuscles of the peacock (*Pavo cristatus*) have not hitherto been described. The object of this note is to place on record the finding of a halteridium in the peacock and its being apparently the cause in this bird of a disease which proved fatal. The finding was made during the course of routine examination of the blood smears submitted by the hospital to the laboratory.

The peacock was brought to the hospital on the 28th of July, 1935. The owner related that the bird was suffering for a week from what appeared to him to be a serious illness, as its mate had died a week ago showing practically the same symptoms. It was off feed, dull, its head hanging down. The feathers were rough and the appearance was very poor. It was passing white and slimy faeces. There was a white sticky discharge from the eyes. Temperature at that time (morning) was 107°F. The body was covered with lice, visible mucous membranes were anaemic and the bird was so weak that it was unable to stand.

EXAMINATION OF THE BLOOD

Examination of the blood smears revealed that a large number of the red blood corpuscles contained intracorpuscular parasites (Plate IV). Such corpuscles, however, did not show any deformity either of the cytoplasm or of the nucleus. Among the corpuscles free from parasites slight anisocytosis was observed.

DESCRIPTION OF THE PARASITE

Repeated minute examination of the blood smears revealed only gametocytes in the red blood corpuscles. No schizonts could be seen. These gametocytes were apparently in different stages of development as shown by the variety of their shapes—Rings, pear, elongated forms, spindles, boomerangs and typical halteridia encircling the nucleus of the host cell. In some, the cytoplasm stained a pale blue with Leishman's stain ; the nucleus was situated in the centre, was large and consisted of a number of chromatin granules stained intensely red and apparently enclosed by a membrane. In these gametocytes the brownish black pigment granules were collected together. These were apparently male gametocytes. In others, apparently females, the cytoplasm took a deeper hue with Leishman's stain and the nucleus was not granular but compact. The number of gametocytes in each corpuscle was variable. Some red cells contained only one mature gametocyte. In others there was a mature gametocyte on each side of the nucleus. These were either both of the same sex or of opposite sexes. In others again there were three or four gametocytes, some of them mature and the other immature. More than four gametocytes in a single corpuscle were not encountered.

TRANSMISSION EXPERIMENT

A fowl was inoculated with the blood obtained from the peacock but daily examination of the blood of the fowl for over a month failed to reveal any parasites. The parasite was thus not transmissible to fowls by blood inoculation.

DISCUSSION.

Further work on this parasite was unfortunately cut short by the owner failing to bring the bird to the laboratory, because, as related by him, the bird died two days after his visit to the hospital. Opportunity is being awaited to pursue this investigation further. In view, however, of the clues yielded by the investigation that has been possible, there can be little doubt that the parasite of the peacock belongs to the *Hoemoproteus* genus of intra-corpuscular parasites of bird malaria. It has been already stated that examination of the blood smears has revealed a notable absence of schizonts which are a characteristic feature of the members—now-a-days regarded as the truly malarial— of the genus *Plasmodium*. The typical halteridium shape, the absence of deformity of the host cells and to some extent the non-transmissibility by blood inoculation are all points in further support of the view that the parasite belongs to the genus *Hoemoproteus*. As reference to the available literature has failed to reveal any mention of this parasite and as there is, now-a-days, among workers on this group a uniform tendency to regard such parasites as distinct species on the simple criterion of host specificity it is considered advisable, tentatively, to hold this species as a new one

until the contrary is proved. From the fate of peacock that came to the hospital and its mate, which showed similar symptoms before death, it is surmised that this parasite can cause an illness which might prove fatal in this bird.

Summary

(1) The occurrence of a species of *Hoemoproteus* in the Indian peacock is recorded.

(2) Pending proof to the contrary, this species is held to be a new one.

(3) It is also surmised that this parasite can cause an illness which may prove fatal in this bird.

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A NEW BLEEDING TRAVIS

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With Plate V and four text-figs.

For both chemical and physiological studies, it is the general custom to draw sample of blood from the jugular vein, after casting and securing the animal. This method has been found to have many disadvantages. The first and foremost is straining the animal to such an extent that the composition of the blood is likely to vary during the time of sampling. Peters and Van Slyke have stated in the Quantitative Clinical Chemistry Interpretations, " There is concentration of Erythrocytes and Haemoglobin during exercise and shock ". Secondly, taking blood samples from pregnant animals is impossible as casting a pregnant cow is not to be thought of. Further, casting a cow even when not pregnant may result in injury to the animal. Lastly blood has to be drawn from animals under digestion experiment. When such animals are cast, it always happens that both urine and faeces are lost and the digestion experiment is spoiled. Waste of time is also a point for consideration when many animals are to be bled on the same day. So, a method had to be devised for drawing blood from the animal in a standing posture. The animal has, therefore, to be firmly secured, so as to prevent it from moving backwards or forwards or from lying down or jumping up, at the same time, giving it the least exertion and pain thereby the chances of accidents are minimised. A detailed description of the travis recently put up in the Animal Nutrition Section at Bangalore satisfies most of the requirements.
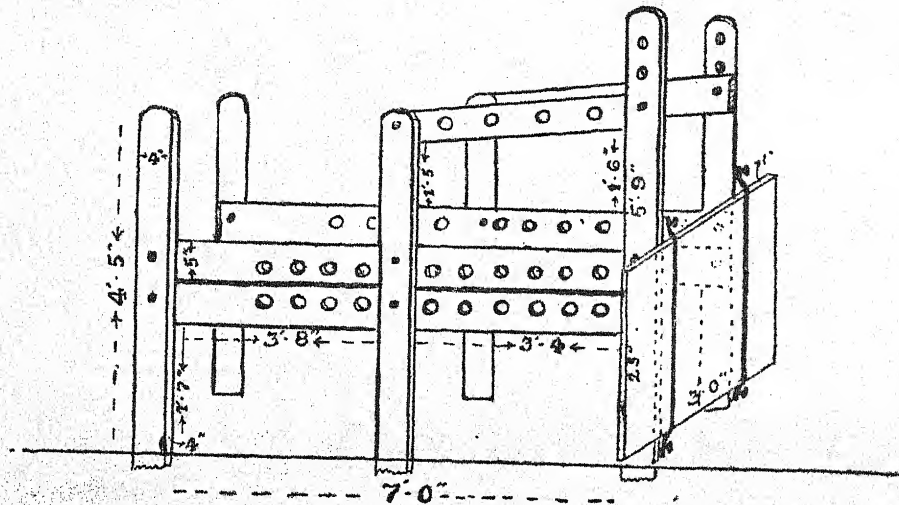
FIG. 1.

BLEEDING TRAVIS.

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The travis which is simply a modified dressing travis as used in the Veterinary Hospitals, consists of (1) Fixed wooden frame, (2) Front plank, (3) Sling pad, (4) Press pad and (5) Neck rope. The sketch (Fig. 1) overleaf is a picture of the wooden frame with all the measurements. The front posts are longer than the back ones, the posts carry two horizontal bars in the middle with holes at suitable lengths to receive cross-bars and the horizontal bars at the top do not reach the last vertical posts. For cross-bars, pieces of G. I. pipings $1\frac{1}{2}$ in are found suitable as they are strong and round. The two front posts carry two iron guards 2 ft. long in front of which one is fixed and the other movable from a horizontal to a vertical position with fixing arrangements. These iron guards hold the front plank in position as shown in the sketch (Fig. 1).

The front plank is $3' \times 1'$ 11″ and is padded on one side and fixed between the guards with the padding inside just before the animal is brought in. This plank apart from holding the animal protects the operator also from injury.
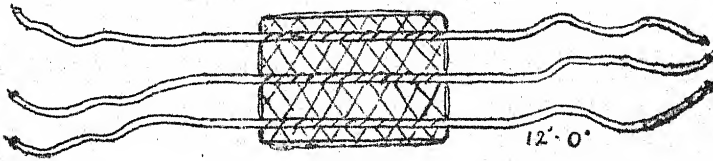
FIG. 2.

Figure 2 is the *sling pad*. This is only a stuffed gunny bag to which three thick ropes (preferably cotton or jute) of sufficient length are stitched on. This padding comes under the animal and with the ropes on both sides tied through holes at the top horizontal plank of the wooden frame, supports the animal as if on slings.
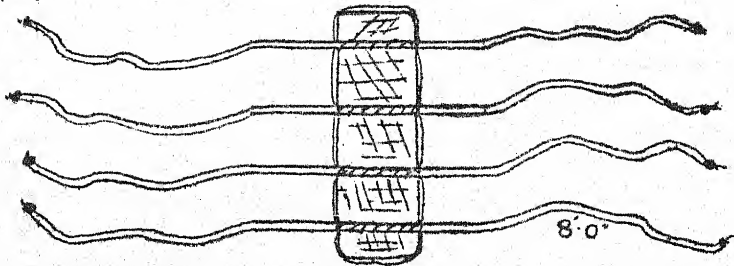
FIG. 3.

Figure 3 is the *press pad*, which is another stuffed gunny bag about half as wide as the one described above to which four similar ropes are stitched on. This cushion is placed over the back of the animal and the ropes on both sides are tied to the bottom horizontal bars.
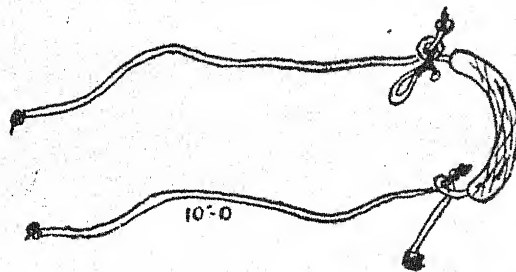
FIG. 4.

Figure 4 is the *neck rope*, which has a neck loop and two long ropes on both sides. The loop enclosed in a padding is fixed at the yoke place and the side ropes are pulled tightly back and fixed to the middle vertical posts.

Besides the above parts, three or four pillow-shaped gunny cushions are made use of, one of which is tied to the post to which the animal's head is turned and fixed, by means of a muzzle rope tied to the post for bleeding from one side. Similarly other cushions are placed wherever the animal gets a moving space and wherever the body comes in contact with the hard wooden frame.

In a bullock the nose string helps a great deal in securing the head but in a cow the nose string is replaced by the muzzle and neck strap which is generally used for horses and the head is strapped to the front post.

All the edges and corners are rounded and smoothened. The sharp edges of the holes are also rounded to prevent wear and tear of the ropes of sling and press pads.

Working of the Travis.—The front plank is fixed before the animal is brought and as soon as the animal is led in, a bar is introduced at the back between the second and the third posts. The holes are to be selected according to the length of the animal. Another bar is placed in front just behind the front plank to prevent the animal from striking with its fore legs, a third one is introduced between the two front posts and this passes above the neck of the animal behind the horns. Now the animal may be considered to have been effectively secured. But it may make attempts to jump or lie down. To prevent that, the sling and the press pads are tied which hold the animal as if in a vice. If it still attempts to move, the neck rope should be tied (this must be the last operation in securing the beast). No further movement is now possible. The pressure applied at the neck by this last operation also makes the jugular vein prominent. Now the animal's head is turned towards the post and fixed over the cushion mentioned above. The entire task of securing can be completed in five minutes. In releasing, the ropes of the sling pads and press pads are untied on one side and the pads are drawn to the other side, and the neck loop is released. Finally the bars behind and above the neck are removed and the animal is then free to move back. Plate V, Fig. 1, is a picture of
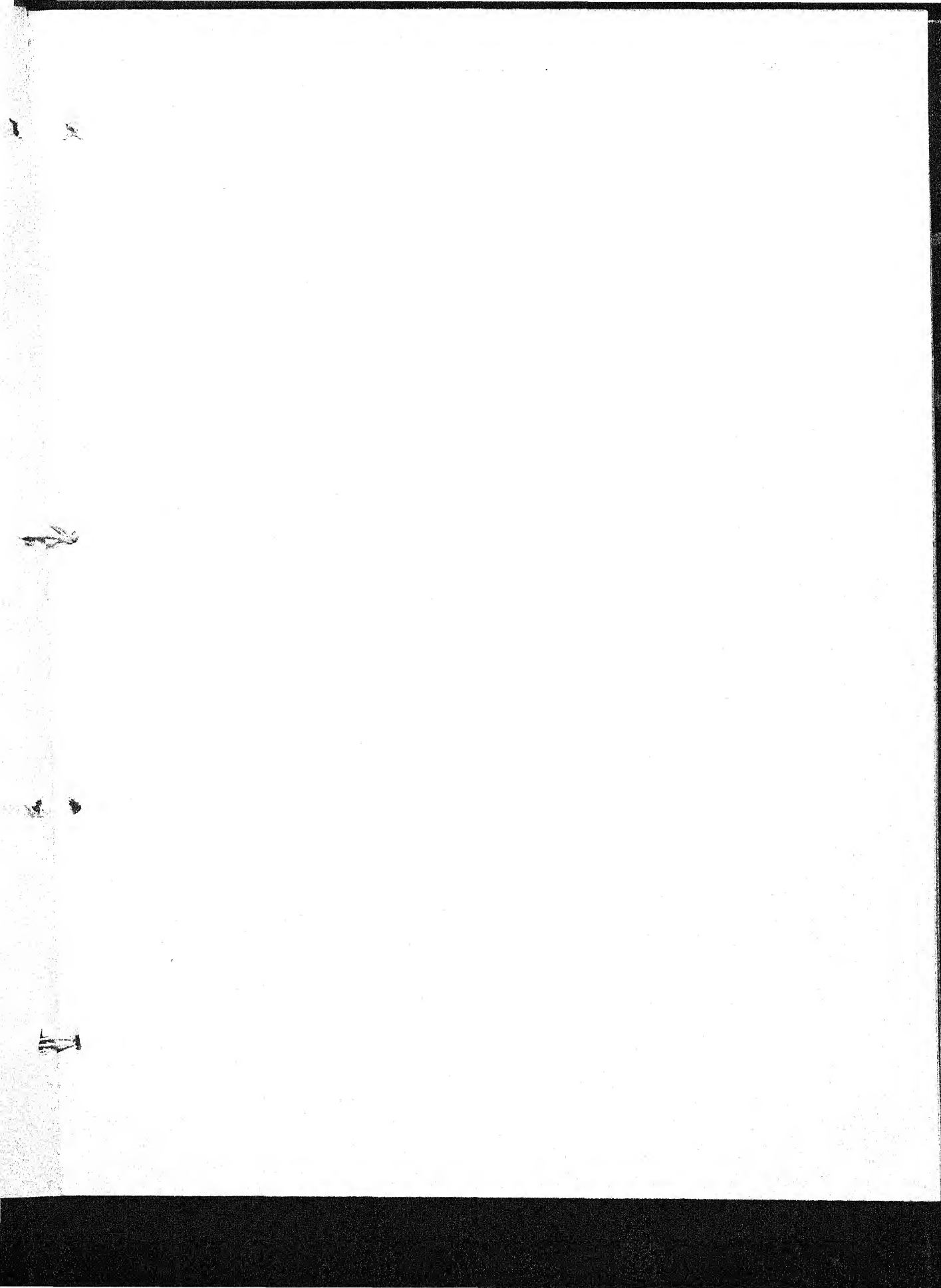
A NEW BLEEDING TRAVIS
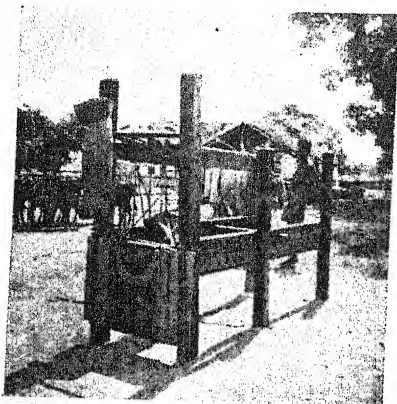
Fig. 1.
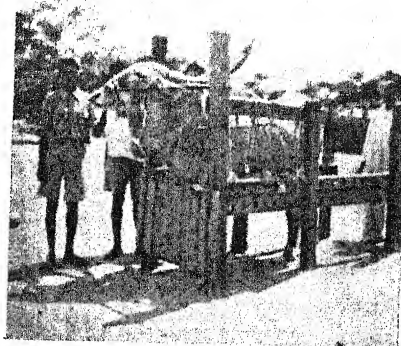
Fig. 2.
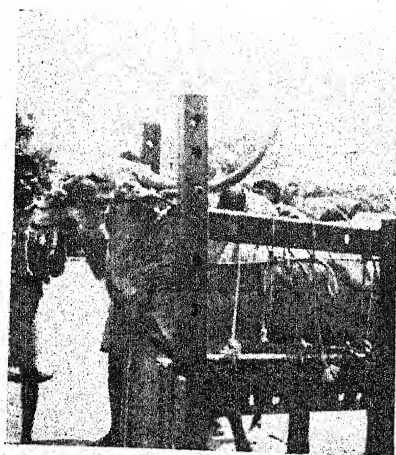
Fig. 3.

Fig. 4.

the travis with all the parts fitted up and ready to receive the animal. Plate **V**, Figs. 2 and 3 are pictures of the same with the animal ready for bleeding. With the devices mentioned above even the most refractory animals can be made to stand still and so far, no case of accident has happened.

Bleeding.—Drawing blood from the jugular vein is known to all professional people. They adopt very elaborate arrangements in sterilisation but those seem to be unnecessary. An ordinary hypodermic needle and a swab of tincture iodine are all that are necessary. The needle is sterilised by swabbing all over with tincture iodine and the seat of operation is also similarly treated. To draw blood the skin at the site of operation is pinched by the left hand and raised, the needle is then thrust into the subcutaneous tissue and after adjusting the length either by drawing out or pushing in the needle, the point of the needle is brought near the projecting vein, then by giving a sharp and gentle push the needle is thrust into the vein which allows a free flow of blood. On the other hand if an attempt is made to thrust the needle direct into the vein, the force exerted to pierce the skin may displace the vein from its position and the needle is likely to miss the vein. The blood is collected by holding a tube below as shown in Plate V, Fig. 4. Blood samples are being taken by this method almost every alternate day and there has not been a single case of any after-effect, not even the slightest swelling at the site of bleeding.

Before concluding it may not be out of place to mention about the quality of blood collected thus. As tincture iodine is freely used, the sample may not be fit for iodine estimations. But absolute alcohol may replace iodine in case iodine estimations are required. As regards sterility, this much can be said that the use of this travis eliminates two sources of outside contamination namely the ground and the bedding. For collecting sterile blood, therefore, this travis offers definite advantages.

I acknowledge with profound gratitude the inspiring guidance given and constant interest taken by Dr. F. J. Warth during the course of this work.

With great pleasure I also thank Mr. N. C. Das Gupta, B.Sc., Assistant to the Physiological Chemist, for his valuable suggestions in perfecting this travis.

SUMMARY

A new travis for drawing blood for experimental purposes from animals in standing posture is described with sketches and photographs,

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SELECTED ARTICLES

DIET AND DISEASE

BY

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The twenty-five years of His Majesty's reign which are now being celebrated correspond remarkably closely with the establishment of a new era in the science of nutrition. At the opening of the twentieth century, attention was being focused on the quantitative relations of the energy exchanges of the body and on the metabolism within the body of the proteins, fats and carbohydrates of the food. The physiologists and chemists working at these problems were making most valuable contributions to the body of knowledge concerning the processes of nutrition, but such contributions were for the most part not of such a nature as to afford obvious clues either to the origin of or to the treatment of disease. The second decade of the twentieth century witnessed the rapid development of the view that the adequate nutrition of an animal depended on the presence in its food of hitherto unsuspected elements. The absence of such essential elements from a diet was proved to result regularly in the appearance of predictable signs of disease and the fundamentally new idea of deficiency diseases became gradually established in current medical teaching.

Before mentioning any of the effects which this new conception of nutrition has had on the problems of the prevention and treatment of disease, it will be useful to hark back to the 'pre-Georgian' era to review the current teaching of the medical profession regarding the relation of diet to disease. The fact that faulty diets are often the direct or indirect cause of disease was of course fully recognised, as it had been for many centuries. But there was little precise knowledge available to enable definite diseases to be ascribed to specific dietetic errors. Over-eating was regarded as predisposing to many gastro-intestinal diseases, gout and raised blood pressure, and under-eating was considered to render the body more liable to invasion by harmful bacteria. The idea of lack of balance between the various classes of foodstuffs, for example, relative excess or deficiency of protein, carbohydrate or fat was looked upon as at least an important contributory cause of disease. In the case of scurvy, it was already taught that the absence from the diet of some principle which was present in fresh foods but not in stale foods contributed largely to the production of the disorder. Otherwise the production of disease by faulty diet was largely related to the presence of toxins, pathogenic bacteria or living parasites in food which had become accidentally contaminated.

With regard to the practical dietetic management of diseases now known to be due to specific dietetic faults, the degree of divergence between the methods of twenty-five years ago and of the present day is distinctly less than would have been expected from a consideration of the knowledge available then and now. Specific remedies in medicine have again and again been discovered empirically and this is true in the realm of dietetics. The treatment recommended twenty-five years ago by at least some enlightened authorities for many of the diseases now spoken of as deficiency diseases would prove satisfactory enough to-day, although such treatment was based on no actual knowledge of the dietetic factors involved. Scurvy was treated by giving fresh fruit and fresh vegetables, rickets and osteomalacia by giving cod liver oil and milk, beriberi by increasing the 'nitrogenous' constituents and diminishing the carbohydrate of the diet—some individuals were even claiming that it could be prevented by adding rice polishings to the diet of highly polished rice which was usually eaten in districts where this disease occurred—and finally pellagra was to be treated by cutting maize out of the diet.

This list of diseases comprises most of those commonly regarded to-day as vitamin deficiency diseases, and there are not lacking critics of modern nutritional research who profess to be un-impressed by its practical value in clinical medicine because many of its obvious applications had been forestalled by empirical methods of treatment. Such arguments fail to recognise the frequency with which valuable methods of treatment fall into disuse or are replaced at least temporarily by worthless imitations when little or nothing is known of the physiological action of the agents effective in alleviating the symptoms of disease. Thus the value of fresh lemon juice in curing scurvy was known in the eighteenth century, but was forgotten for long periods, and far inferior therapeutic agents were sanctioned by high authorities as late as the present century. Similarly the value of cod liver oil for the cure of rickets had been appreciated for many years before the discovery of vitamins, but this did not prevent the subsequent recommendation of inert vegetable fats as satisfactory substitutes.

It is not argued that empirical treatment has no stable foundation in therapeutics, for clinical medicine still makes use of many old-established therapeutic measures which have as yet no scientific basis. But it is no less certain that the scientific demonstration of a definite cause for a disease offers the best chance for the discovery of satisfactory preventive and curative treatment, and when the cause is proved to be a comparatively simple deficiency in the diet, there should be no excuse whatever for allowing such knowledge to sink into oblivion.

It is now proposed to show how the conception of food deficiencies as a cause of disease has developed during the past twenty-five years. The great stimulus to the remarkable activity shown in this field of medical investigation during this period was undoubtedly the discovery of the vitamins, but the success rapidly

attained by those engaged in studying the effects of vitamin deficiencies encouraged the investigation of the effects of other deficiencies, for example, deficiency in the mineral components of the diet, with the result that in such fields also many observations of the greatest importance have been made which have already proved invaluable alike in clinical and in veterinary medicine.

The period of nutritional research which we are surveying had already been heralded by isolated suggestions regarding the importance of dietetic factors other than proteins, fats, carbohydrates and minerals salts for the maintenance of health. Already in 1897, Eijkman had published his experiments on the production of beriberi, which led ultimately to the discovery of the antineuritic vitamin. Hopkins had stated in 1906 his conviction that scurvy, rickets and probably other states of ill-health were caused by unknown dietetic errors the nature of which was bound up with a defective supply of obscure food components. In 1907, Holst and Frolich paved the way for the identification of the antiscorbutic vitamin by producing experimental scurvy in guinea pigs. But it was not until Hopkins had demonstrated in 1912, the fundamental importance of accessory factors in the diet for securing normal nutrition that the idea of specific food deficiencies as a cause of disease began to gain any general acceptance.

It is common knowledge that since Hopkins's original announcement the number of accessory food factors or vitamins generally recognised as being concerned in animal nutrition has been steadily increasing, though not all of them have been shown to be concerned in the production of human deficiency diseases. By the end of the first decade of King George's reign, overwhelming evidence had been produced to show that beriberi was produced by deficiency of the water-soluble antineuritic vitamin, Xerophthalmia by deficiency of a fat-soluable vitamin, rickets by deficiency of the same or a similar vitamin and scurvy by deficiency of the water-soluble antiscorbutic vitamin. It is not possible to trace in detail subsequent investigations which have led on one hand to the chemical indentification of many of the vitamins and on the other to some understanding of their physiological action. It must suffice to point out how such knowledge has been applied to the prevention and treatment of disease.

In countries such as Great Britain, frank vitamin-deficiency diseases, with the single exception of rickets, are uncommon, but there is increasing evidence that partial deficiencies of vitamins, particularly during the period of growth, are often responsible for sub-optimal physical development, imperfections in the structure of bodily organs and tissues, lowered resistance to certain infective diseases and many vague subjective and objective symptoms of ill-health. It is almost certain, for example, that mild degrees of skeletal deformity caused by faulty feeding in childhood help to raise maternal mortality in childbirth by increasing the mechanical difficulties of labour. The low resistance of the teeth of large sections of our population to decay with its many sequelae of chronic disease and ill-health is due in

large part to faulty development of the teeth during the early years of life brought about by dietetic faults, of which vitamin-D deficiency is the most prominent. Similarly it has been shown that, in dogs at least, the tendency to the development of pyorrhoea alveolaris in adult life is largely determined by the supply of vitamin A which was available during the period of growth. These few illustrations must suffice to indicate that the original conception of vitamin-deficiency diseases has extended its boundaries to include a variety of diseases attributable to past dietary deficiencies, preventible by suitable feeding during the period of growth.

Similar results have been achieved during the period under review by the study of specific mineral deficiency diseases. Prominent among those mineral elements deficiency of which in the diet may lead to recognisable symptoms of disease are iron, copper, iodine, phosphorus, calcium and magnesium. Lack of sufficient iron in the food has been shown to result frequently in the development of anaemia. Such nutritional anaemia is particularly common in infants, and is accounted for by the fact that milk is a poor source of iron. The recognition of this form of anaemia has proved to be of considerable practical importance because it is often associated with an increased susceptibility to many of the common complaints of infancy and can be corrected with great ease. The enormous amount of work that has lately been carried out on the relation between iodine deficiency and the development of goitre has not yet completely solved the problem of the causes of thyroid enlargement but it has certainly provided very successful methods of wholesale prophylaxis in districts where goitre is endemic. Lastly may be mentioned the revolution in the cattle-rearing industry of South Africa which has followed the discovery of phosphorus deficiency as the immediate or ultimate cause of serious losses in this branch of agriculture.

There can be no question that already the modern conception of nutrition has produced practical results of immense significance in both preventive and curative clinical and veterinary medicine. The results are not confined to the prophylaxis and treatment of diseases which arise as the result of actual deficiencies in the food supplied. They have already been extended to include the treatment of diseases in which defects in the absorption or utilisation of particular food elements rather than faulty diets are responsible for the development of co-existing signs of deficiency diseases. The term ' secondary deficiency disease ' is now being used to distinguish this particular class of nutritional disorder. In this group might well be placed that once fatal malady pernicious anaemia, which the brilliant researches of the past decade have shown to be amenable to simple dietetic treatment, although there is no evidence that dietetic errors play any part in its causation. As more and more precise information is gained concerning the intimate processes of metabolism within the body, there should be

increasing opportunities of preventing and successfully treating disease by adjusting the diet to influence those metabolic processes which may produce the symptoms of disease when they deviate from their normal course. This has long been one of the aims of clinical medicine ; its ultimate realisation has surely been brought one stage nearer fulfilment by the nutritional investigations of the past twenty-five years.

VIRUSES AS THE CAUSE OF DISEASE

BY

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At the end of the nineteenth century, a new category of infective agents was discovered which are now classed as viruses, in the modern sense of the word. The chief property which unites them, and by which they have been distinguished from previously known minute parasites, is the extremely small size of their component particles, since these are smaller than bacteria, and many of them are not visible even with the highest powers of the microscope. Until quite recently, it was customary to speak of all viruses as invisible, but in some cases the minute granules of which the virus appears to consist can be clearly seen, when stained, by direct microscopic observation ; but in most cases they cannot be distinguished by their shape, but only by their uniformity, numbers and their source in special parts of the diseased tissues. The recent investigations by Barnard with the ultra-microscope and photo-micrography by ultra-violet light have added to our knowledge of their size and form.

A special feature by which the invasion of the cells of the host by many viruses can be recognised is the occurrence of ' cell inclusion bodies '. These forms of which there may be one or more in a single cell vary in size and may be larger than the nucleus. Opinion as to their nature has undergone various vicissitudes. After their discovery, when they were at first hailed as protozoal parasites, they were for long regarded as merely reaction products of the cell protoplasm to the presence of the virus, a position now favoured for the ' inclusion bodies ' associated with virus diseases of plants. More recently it has been shown that in some virus diseases of animals these ' inclusion bodies ' consist of masses of the minute filterable forms of ' elementary bodies ' held together by a soluble matrix. These bodies have been most completely and fruitfully studied in fowl-pox, small-pox, vaccinia, ectromelia—a disease of mice—and psittacosis, the infective disease of parakeets which also attacks man.

The list of diseases of man and animals due to filterable viruses is continually being increased and considerably more than fifty are now known ; their study has been intensified and especially productive during the last fifteen years.

The original recognition of the existence and importance of these agents was due to the use of earthenware and porcelain filters which retained the smallest bacteria. When tissue extracts or secretions from an infected animal were passed through such filters, the filtrates were shown to be infective and capable of reproducing the disease in a fresh animal ; this process could be repeated indefinitely, proving that the active agent multiplied in the animal body and was not merely a chemical substance of toxin. The virus of foot-and-mouth disease, the first

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shown to cause a disease of animals, was discovered in 1898 by Loeffler and Frosch. It passes through finer filters than any other known virus, so that no question of visible particles has arisen, since massed granules or inclusion bodies have not been observed. The most essential qualifying characteristic of a virus has ever since been its filterability.

Most viruses remain active after being dried over sulphuric acid, and some are more resistant to alcohol and certain other disinfectants than bacteria. Many viruses are present in the tissue juices in high concentration, and such suspensions in liquids can still prove infective when diluted 1 in 10^5 or 1 in 10^6. The resemblance in many respects of a virus to an excessively minute bacterium has led to the belief in their similar nature which is now held by most pathologists. It must be admitted, however, that part of the argument is based on analogy, since viruses cannot be subjected to the same tests as bacteria to prove that they are living agents causing disease.

Besides the small size of the ultimate particles of a virus and the resulting absence of a recognisable differentiating morphology, there are certain other peculiarities distinguishing these two classes of agents. Bacteria, with few exceptions can be propagated on sterilised artificial culture media, and can be obtained in pure culture by the method introduced by Koch of selecting single colonies grown on a solid sterile medium. By this means their infective and other activities can be examined without the risk of contamination with substances derived from the host. A virus, on the other hand, in most cases requires the presence of living cells of the host to enable it to multiply, and it often appears to grow only or chiefly inside the cells of the animal tissues. Artificial culture of a virus can, however, very often be maintained in pieces of animal tissue kept alive and growing apart from the body. The virus of fowl-pox, vaccinia or vesicular stomatitis of the horse and of some other diseases can also be propagated in the living embryo in an incubated hen's egg.

The fact that tissues of a host are needed to enable viruses to multiply has led to the suggestion that the virus may not necessarily be alive but may only serve as a stimulus to the host cells, causing them to reproduce the virus, and that the particles seen in a suspension containing a virus and indeed the virus itself are really products of the host.

This suspicion has been especially strong in the case of the infective transmissible sarcoma of fowls described by Rous, which can be reproduced by injecting a filtered cell-free extract of the diseased tissues into a normal fowl. The resemblance of avian sarcoma to other virus diseases extends to the recognition by Ledingham and Gye, by the use of the high-speed centrifuge, of minute particles resembling those of other viruses. The nature of these particles was, moreover, confirmed by their reaction (agglutination with the blood serum of animals which had been injected with the sarcoma), in the same way that similar reactions have been demonstrated with the elementary bodies from other virus diseases.

The resemblance in structure and behaviour of the sarcoma of fowls to the malignant growths of mammals gives rise to hesitation before admitting that it is caused by an extrinsic virus, since mammalian malignant tumours have never been found to yield an infective cell-free extract and have been usually regarded as due to intrinsic tissue changes, though both mammalian and avian sarcomata can be induced by external physical and chemical irritants, such as tar and certain other substances.

If, therefore, an extrinsic virus is one of the essential causes of fowl sarcoma, it must already be present in every susceptible fowl. The view that viruses which produce disease are not essentially invading parasites, but are produced by the host, is opposed by the regularity with which diverse viruses can be propagated in the same kind of animal, and by the fact that the same virus may infect several widely different species. For example, there are three distinct types of foot-and-mouth disease virus which produce apparently identical symptoms in animals and can only be distinguished by the fact that any one does not protect an animal against infection with the other two ; nevertheless, each virus maintains its identity whether propagated in the cow, pig, guinea pig, rat or hedgehog. It is difficult to see how this could happen if the virus were produced by each species of mammal from its own tissues.

Another filterable agent, in many respects resembling the virus of an animal disease, is the bacteriophage which was independently discovered by Twort and D'Herelle. The effect of a drop of a suspension of bacteriophage added to a young liquid culture of susceptible bacteria is that the latter are dissolved and a large amount of fresh phage is produced. The bacterium-free filtrate of the liquid culture may often be diluted ten million times and still the same effect be produced by a drop as by the original suspension.

D'Herelle and many other bacteriologists believe that the phage is a living agent which infects young growing bacteria, multiplies in their interior, and is set free when the bacteria die and break up.

Phage, though destroyed at a temperature of 70° to 75°C., as a rule survives at 60° to 65°C., when the bacteria with which it is associated are killed ; it also resists drying and is remarkably resistant to the action of alcohol, acetone and chloroform. D'Herelle considers that all strains of phage are really one though different strains become adapted to different bacteria, but more probably many phages when first obtained are a mixture of distinct races, and most filtrates containing phage are in the first instance derived from sewage or faeces, containing a great variety of bacteria.

Some strains of bacteria harbour a phage although apparently insusceptible to its destructive action. The activity is only manifested when a filterate is tested on another susceptible strain. Thus many bacterial cultures have been shown to produce phage, though the presence of phage is not apparent and may not even

have been suspected. This phenomenon suggests the original production of the phage by an uninfected culture, but it may merely be another instance of an apparently normal organism ' carrying ' a parasite ; many parallel cases are known of animals and plants ' carrying ' infective agents whilst themselves unaffected. De Jong showed that cultures of certain sporing bacilli which produce a phage may still be ' lysogenic ' after being heated at 100°C. for five minutes, whereas the free phage is killed at 70°C. for five minutes. When the spores germinate the phage is again liberated. This experiment suggests that the phage is preserved by its inclusion in the resistant spore, and this evidence of its derivation from the germinating spore *de novo* is not conclusive.

The chief reason for doubting the living nature of some phages and certain viruses is the very small size of their filterable particles which makes it very doubtful whether they can have a complex composition resembling that of other living things.

Different strains of phage are very unequal in their filterability ; some have relatively large particles with a diameter about half those of vaccina, while others pass through very fine filters, like the virus of foot-and-mouth disease, for which the diameter is estimated at about one tenth of the coarser phages.

The uniform and carefully graded collodion membranes introduced by Elford, of which the average pore diameter can be calculated, enables much closer estimates to be made than formerly of the size of particles which just pass or are just withheld. The size of the particles of some viruses has also been calculated, especially by Bechhold, by their rate of deposition when centrifuged at 10,000—15,000 rev. per min.

It has been possible to purify virus particles by first filtering and then centrifuging at high speed, washing the deposit and again centrifuging, as has been shown by Ledingham.

By the new collodion ultra-filters the diameter of the particles of different viruses has been estimated to vary from 200 mμ to 150 mμ for vaccinia to about 8-10 mμ for foot-and-mouth disease (μ=mikron=1 thousandth of a millimetre ; mμ=one thousandth of a mikron). It is difficult to understand how with such dimensions they can have a composition of sufficient complexity to consist of living matter. For comparison, the smallest bacteria have a diameter of $1 \cdot 0$—$0 \cdot 5\mu$ and the egg-albumen molecule has been estimated at $4 \cdot 34$ mμ diameter.

Doerr, in a recent treatise, while granting that some viruses have been shown to be living denies the possibility of life in those of the smaller dimensions. It so happens that the viruses of foot-and-mouth disease and louping-ill which are among those with the smallest particles, exhibit all the typical essential characters of viruses both *in vitro* and in the animal body, though causing very different diseases and having very different ' life-histories '.

The exact and quantitative experiments with filters have been made possible by the high concentration in which certain viruses occur, and by opportunities for determining the presence of the virus in different dilutions by inoculation of susceptible small animals. The dilution of some fluids containing virus from the animal body can be carried to 1 in 10^6 or even higher when dealing with foot-and-mouth disease, vaccinia and some other diseases without depriving them of infectivity.

The quandary arising from the very active and apparently vital functions of virus particles in spite of their small size raises the question whether the accepted definitions of life are universally applicable or whether some intermediate state between what is called living and dead matter may not exist, as has been suggested by Boycott.

Of the functions usually postulated for a living organism, assimilation appears to be the most characteristic and indispensable. It is reasonable to assume that the metabolism of an organism would be much simplified if it existed in a circulating medium which provided a constantly changing supply of materials resembling its own components, such as might be afforded for an obligatory parasite living inside the cells of its host. Such an existence would have very different requirements from a truly independent life.

Virus diseases are transmitted from one animal to another by very varied means. Some, like canine distemper and certain influenza-like diseases of man, by droplets in the breath, others like yellow and dengue fevers by the bites of insects, others like louping-ill of sheep by the bites of blood-sucking ticks or of mites; again, the bite of the mammalian host is the usual mode of infection with rabies, but for many others the method of transmission is still uncertain. In these respects they do not differ from diseases due to bacteria.

It is characteristic of many diseases that, although the initial infection is caused by a virus, many of the symptoms and complications are due to secondary infections with bacteria, and this is notably the case in the influenza-like group in man in swine fever, canine distemper and swine influenza.

A virus may become remarkably adapted and sometimes permanently attenuated, when transferred to a new host, as is well instanced in the change of the virus of small-pox to vaccinia in cattle and rabbits, and of the rabies virus in the rabbit.

The period of resistance shown by the host following an attack of disease is sometimes very prolonged even life-long after small-pox, varicella, yellow fever and canine distemper, but in some other cases the protection afforded is of comparatively short duration, in foot-and-mouth disease usually for one to two years, whereas frequently recurring attacks due to the virus of *herpes labialis* are common.

This immunity is to a great extent due to the production in the animal body of 'antibodies' which can be found in the blood serum of recovered animals just as occurs after bacterial infection. These antibodies can often be demonstrated by the formation of a precipitate or by the agglutination of the virus particles when a suspension of the elementary bodies is mixed with the serum, or by the neutralisation of the virus by the serum when both are injected into an animal. These phenomena are of the same kind as the precipitation occurring when the blood serum of an animal which has been inoculated with a foreign protein (antigen) is mixed with the same protein *in vitro*, and are not peculiar to true infection.

It is not intended here to do more than refer to the enormous and increasing number of filterable viruses known to cause infective disease in plants and found in their juices. These, like mosaic disease of tobacco, spotted wilt of tomato and crinkle and leaf-roll of potato may cause very serious disease, or in other cases may be present throughout the plant without producing any visible effect, as in some infections of the potato.

There is good evidence that two viruses may co-exist in the same plant, and as a result the symptoms may be either much more or much less severe than when either virus is present alone.

Some viruses are transmitted by insects such as aphis or thrips, while others pass by unknown means. In some of these diseases of plants peculiar 'inclusion bodies' are found in certain cells, but their relation to the virus is undetermined. It is known that these 'bodies' as well as some of the symptoms due to a virus can in special cases be imitated by the addition of certain inorganic salts to the soil, but the disease is not then transmissible. Some plant viruses are highly resistant to drying chemical action and alcohol, and in many ways the viruses of plants resemble those of animals.

The problems which the behaviour and properties of viruses raise are of great practical and theoretical interest, and are by no means yet solved.

INHERITANCE AS A FACTOR IN POULTRY DISEASE*

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During the past three decades the poultry industry in the United States has undergone tremendous changes. From the family flock basis, where birds were kept principally as a source of meat and eggs, it has gradually changed until today poultry keeping in its various aspects is the primary enterprise of thousands of farmers. With this change many problems that were not of great concern in the family flock have developed. Of first rank among these problems is that of flock mortality, which instead of being on the wane seemingly is becoming a more serious menace year by year.

SIGNIFICANCE OF DISEASE LOSS

The seriousness of mortality in commercial flocks has been emphasized in economic studies of poultry farming in various states. In New Jersey in 1915-16 the mortality was 7 per cent [App et al., 1919]; in Oregon in 1926-28 the average mortality was 13 per cent [Scudder et al., 1931]; 16·9 per cent in New Hampshire in 1929-30 [Woodworth and Reed, 1932]; 21 per cent in Utah 1929-30 [Thomas and Clawson, 1931]; 27 per cent in New York in 1930-31 [Misner, 1931]; and 21 per cent. in Iowa in 1933 [Vernon and Whitfield, 1933]. In egg-laying contests mortality has been equally severe. Stafseth and Weisner [1931] observed 18·98 per cent mortality among 10,000 pullets in eight successive years at the Michigan contest. Furthermore, they point out that poultry diseases are increasing and that the losses during the later years of the contest were greater than in the earlier years. In the Vineland egg-laying contest Black [1933] reports mortality rates of 24·51, 29·75 and 23·36 for the years 1929-30, 1930-31 and 1931-32, respectively.

The seriousness of such losses to the industry cannot be over-emphasized. Obviously if the poultryman is to succeed he not only must raise good pullets but he must keep them alive. Lippincott and Card [1934] state that pullet mortality and, to a less extent mortality in older hens, is one of the most serious problems confronting poultrymen. Furthermore, they say that while good sanitary measures have enabled flock owners to bring mortality in growing chicks reasonably well under control, that the problem of reducing or preventing mortality in laying stock is still to be solved. They believe that one of the most promising methods of

attack is the breeding and selection of resistant strains, though they point out that progress is likely to be slow until the vital significance of the problem is generally realized.

Flock mortality is due, obviously, to diseases of various types and any programme for disease control must recognize and take into consideration the different kinds of diseases responsible for this mortality.

METHODS OF CONTROL

Three possible methods of disease prevention and control are available to poultrymen. The first, and perhaps the foremost in importance, is proper hygiene and sanitation for the flock. A second method is through the use of various therapeutic agents and the third method is genetic. The genetic or breeding method is concerned with the development of strains free from defects and disturbances of function, and, likewise, with the creation of strains having a high natural resistance to parasites and other infectious agents.

The genetic method of disease control, therefore, implies two rather distinct categories of disease, namely the non-infectious or those diseases due to derangements of structure or function, and the infectious. It is the purpose of the writer to review the present status of our knowledge of the genetic aspects of disease and this review will be presented under the above general headings. Certain diseases, such as neurolymphomatosis, the etiology of which is still undetermined, will be considered separately.

THE NON-INFECTIOUS DISEASES

Non-infectious disturbances involving deviations from the normal or desirable type in structure and function have been shown to be hereditary in many cases, and it is probable that most diseases of this sort will be shown to have some hereditary basis. Examples of such inherited defects in poultry are the creeper fowl [Landauer and Dunn, 1930], congenital loco [Knowlton, 1929], congenital palsy [Hutt, 1934], dwarfism [Mayhew and Upp, 1932], and stickiness [Byerly and Jull, 1932]. Since many of these conditions are lethal or else produce some other serious detrimental effect in the affected individual they are responsible for reduction in reproductive efficiency and a corresponding economic loss. Hence it is essential for the welfare of the poultry industry to prevent the wide-spread dissemination of such genes through the population. The eradication of such diseases is purely a genetic problem, and any intelligent plan for disease control in the poultry industry certainly must consider the breeding programme if such defects are to be eliminated.

While clear-cut genetic evidence is lacking, it is probable that numerous weaknesses of the reproductive system, which result in much pullet mortality, are hereditary. A study of the causes of mortality in any of the egg-laying contests will show that much mortality is due to such causes as prolapsis of the oviduct, rupture of the oviduct, and to other causes in which an infective agent

apparently is not involved. In their attempt to increase production many poultrymen have neglected to take into consideration the inherent stamina of their birds, which seemingly is a very vital factor if high egg production is to be maintained over long periods of time with little mortality. Dunnicliff [1914] has pointed out the importance of this factor in his analysis of the Hawkesbury egglaying contest. He says that some strains show much greater susceptibility to ovarian weaknesses than do others. In the Hawkesbury contest the competition committee adopted the policy of awarding a special trophy to the pen laying the most eggs without the replacement of a bird. Some high producing pens went through three years without the replacement of a single bird. As a result of his analysis Dunnicliff concludes that breeding for constitution will have to be considered much more closely than in the past, and he is hopeful that close attention to the salient points of breeding will result in eventual solution of this most difficult problem.

Kennard [1933] has demonstrated the importance of selection based upon the simple expedient of using hens as the progenitors of the pullets to be retained as layers. By this method he was able to bring about an appreciable decrease in pullet mortality, and he attributes a part of the increase in pullet mortality, observed generally by poultrymen to the practice of selecting their breeding stock from pullets. As a permanent solution of the problem of pullet mortality, he advocates careful breeding and selection, combined with a type of management that will insure that young birds reach maturity free of parasites and unexposed to diseases.

While it would be rash to assume that most pullet mortality is due to hereditary weaknesses, sufficient evidence has been advanced in man and other animals [Pearl, 1928], as well as in poultry, to show that longevity and strong constitution are inborn traits of certain families and strains. It would seem inevitable, therefore that any long-time plan of poultry improvement, if it is to be economically successful, must consider the elimination of defects and the selection of strong hardy strains as vital parts of its programme. Certainly the genetic method of control is a most important one for diseases of this sort.

THE INFECTIOUS DISEASES

An infectious disease, in contrast to the non-infectious, requires the co-operation of two agents, the host and the parasite for its expression. That heritable differences in resistance to such diseases exist has long been known, but until the last ten years most of this evidence was statistical in nature, or was confined to the demonstration of species and racial differences. In the last decade, however, several investigators have shown that heritable difference in resistance to bacterial diseases are common within the species. In poultry, Roberts and Card [1926] have demonstrated the effectiveness of selective breeding upon resistance to pullorum disease, and Lambert [1932] has shown similar results for

fowl typhoid. Five generations of such selective breeding decreased the mortality from fowl typhoid from 85 to 10 per cent. Roberts [1932] has recently reported that the resistance of the pullorum stock has been maintained for seven consecutive generations, the respective mortalities for the selected and unselected stocks being 35 and 73 per cent. Furthermore, both Roberts and Card [1926] and Lambert [1932] have shown that strain differences in resistance to both these diseases exist in unselected flocks.

In other species, also, clear-cut demonstrations of the role of heredity in disease resistance have been shown. Schott [1932] and Webster [1933] were able to build up remarkable resistance to typhoid-like infections in mice, Irwin [1929] to the *Danysz bacillus* in the rat, and Manresa [1932] to infectious abortion in rabbits. Other demonstrations of the part of heredity in infectious disease have been made by Frateur [1924], the Hagedoorns [1920] and by Gowen and Schott [1933].

That a passive transfer of immunity was not responsible for the increased resistance in the selected stock has been amply demonstrated by Irwin [1929], Lambert and Knox [1932], Schott [1932] and Gowen and Schott [1933]. The resistance has been shown to be transmitted by the male as well as by the female. In addition, Lambert [1932] and Roberts and Card [1927] have pointed out that in stocks selected for resistance to fowl typhoid and pullorum disease, chicks hatching from the eggs of females who remain carriers of the pathogenic bacterium are neither more nor less resistant than chicks produced from the eggs of non-carrier females of these stocks.

The hereditary basis of resistance and susceptibility to infectious diseases appears, from most of the results so far available, to be complex, probably being dependent upon multiple factors. This, obviously, complicates the problem of using the genetic method for producing commercially desirable lines of resistant birds. It must be remembered, however, that very little research has been done on this problem and that a blanket rejection of a method on the basis of difficulty of accomplishment is unjustified. Certainly poultry furnishes the greatest opportunity for using the breeding method of any of our domestic animals of great economic importance. In poultry, large progenies are available and this permits of selection of large numbers and of elimination on a large scale. In addition, the cost of the individual animal is low, a factor which often makes the use of therapeutic agents, as costly vaccines, economically unjustifiable.

The whole question of the use of the breeding method in the case of infectious diseases is one upon which more information is sadly needed. Cole [1932] has pointed out that the ease with which preventive and therapeutic measures can be applied has led to a neglect of any natural resistance present in our flocks, a method of control which would be more satisfactory if naturally resistant stocks can be developed.

As pointed out by Cole, if it proves impracticable to develop high resistance in commercial flocks by selection, the same general result might be attained by developing special resistant flocks in which most of the genes had been made homozygous by selection and inbreeding. Such flocks could furnish sires to commercial poultrymen to be used for top-crossing on their stock. Such a programme should raise the general level of resistance in flocks. As the general level of resistance rose the need for treatment and other protective measures would be correspondingly lessened.

The present commercial set-up in poultry with the wide distribution of chicks through hatcheries should furnish ample opportunity for the development of such a programme. Of course, any attempt to develop disease-resistant lines should first be made for those diseases which cannot easily be brought under control by other means, or in which the seriousness of the disease would justify large efforts on all methods of control.

Diseases of uncertain etiology

Neurolymphomatosis, the etiology of which is still undetermined, is undoubtedly the disease of greatest economic importance in poultry, in which no preventive or curative therapeutic treatment has been found. It would seem from the studies of Biely and his co-workers [1932, 1933] that the breeding method may be the most important means for controlling this disease. They have demonstrated that the differences in incidence of paralysis in different groups and families of chickens are highly significant, and in the later report they suggest that the difference between resistance and susceptibility is possibly controlled by one pair of genes. While such a conclusion is somewhat hazardous in view of the difficulty of reproducing the disease, the results certainly point to the desirability for further research.

The results of Kennard [1933] also suggest the probability of inherent differences in resistance, and of the importance of selection against paralysis in a flock. That strain differences in resistance occur is also recognized by pathologists who have given much attention to this disease [see Patterson et al, 1932].

Discussion

The examples cited in the foregoing pages clearly emphasize the potential importance of the hereditary factor in disease. While little of practical importance in the way of disease control has been accomplished by use of the genetic method, it need only be pointed out that little attempt has been made to use this method in a systematic way. Further experimentation is greatly needed, and it is unfortunate indeed that our laboratories of poultry research have so generally overlooked this problem. The incomplete success of present methods of disease control and the importance of the breeding method, at least for some types of disease, certainly justify its inclusion in the research programme of many institutions. What is badly needed is a concerted research programme which has

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as its aim the collecting of information and statistics on all phases of disease, and in which all possible methods of disease control are given due consideration. Such a programme obviously, is too large for any one institution, but is it too much to hope that a co-ordinated programme can be evolved in which the workers of many institutions will co-operate in the solution of the problems presented by disease ? Certainly it would seem the industry is sadly in need of such a programme.

Some possible steps that might be included in such a research programme to throw further light on the use of the genetic method in disease control are as follows :

A determination of the value of breeding only from hens, namely second year and older birds, upon the basis of their pullet performance. Such hens not only should have survived the pullet year with a good production record and a clean bill of health, but they should come only from those families having the best records in these respects. A breeding programme based upon this policy should produce hardier and more disease-resistant progeny.

The careful collection of disease statistics upon many strains of birds. Meagre studies indicate that marked differences in resistance to disease as well as in general stamina are strain characteristics. At present our information on this question is woefully inadequate.

Once desirable strains of birds are isolated, to test these strains in intercrosses, with the aim of combining the desirable traits of both strains in a new strain or strains. Sufficient evidence is available to indicate that some crosses will produce first-generation hybrids of high merit, both in production and in general stamina.

The development of inbred lines from the more desirable strains which can be used to furnish sires for topcrossing and as foundation stock for intercrosses. Such inbreds obviously should be free from undesirable defects and other physiological weaknesses. From the top crosses and intercrosses continual effort should be made to develop better inbreds.

A revamping of the objectives of our laying contests and R. O. P. programmes with more emphasis on determining the inherent stamina of various strains, as well as high production and other desirable characteristics. Properly conducted laying contests should furnish a source of invaluable information on all phases of disease, and upon the inherent potentialities of many strains of birds. The number of such properly conducted tests should be increased.

Many of these suggestions, obviously, are suitable only for long-time programmes. However, the menace of disease to the success of the poultry industry, and the apparent increase in the incidence of disease, in spite of sanitation and the use of therapeutic measures, would indicate the need for the adoption of a long-range programme, inclusive in its scope and vigorous in its prosecution.

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THE RELATION OF VITAMIN-D TO CALCIUM AND PHOSPHORUS RETENTION IN CATTLE AS SHOWN BY BALANCE TRIALS

BY

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The fact that a calf has a definite vitamin-D requirement and suffers from a deficiency of this factor in its ration has been demonstrated by the published work of Rupel, Bohstedt and Hart (1), Bechdel, Landsburg and Hill (2), Huffman (3), and by unpublished work of Gullickson (4) in which experimental "rickets" developed when vitamin-D was withheld and disappeared when this factor was added to the ration. Rupel, Bohstedt and Hart (1), and Bechdel, Landsburg and Hill (2) have reported an increased ash percentage in certain representative bones, and an improvement in the concentration of calcium and inorganic phosphorus in the blood plasma following vitamin-D therapy of animals suffering from experimental "rickets," which gives indirect evidence of a beneficial effect of vitamin-D on the calcium and phosphorus retention, but studies to directly measure this relationship have not been made.

EXPERIMENTAL METHODS

Ten-day mineral balance trials were employed for directly measuring the calcium and phosphorus retention. The mineral retention of normal calves was obtained first to be used for comparative purposes. Young, growing calves were then placed on basal experimental rations relatively low in calcium and phosphorus content, and deficient in vitamin-D. Prairie hay was used as roughage for one group, and beet pulp for the other. The concentrates included corn, corn gluten meal, oats, corn starch, and a little wheat bran. For some animals the basal rations were supplemented with calcium, as calcium carbonate, and/or phosphorus, as monobasic sodium phosphate, and /or vitamin-D, as viosterol (250 D), or sunshine. Balance trials were run periodically to follow any changes and to note the effect of the various experimental schedules on the calcium and phosphorus retention of the calves. On all calves that developed a vitamin-D deficiency as indicated by a rachitic syndrome and a subnormal concentration of calcium and inorganic phosphorus in the blood plasma, particular attention was paid to obtain a balance trial while the calf was still eating well, but after the deficiency was well established. Vitamin-D was then added to the ration, and after the lapse of a reasonable length of time, another trial was run.

Except when the experimental schedule required exposure to sunshine the animals were kept indoors in box stalls. A large indoor pen was available for additional exercise. Feeding was done individually and an accurate record kept of actual consumption. All feeds were analyzed for nutrient and calcium and phosphorus content before they were included in the ration. Weights and measurements of height at withers were taken periodically and the rations adjusted at 30-day intervals to furnish somewhat more than the nutrient requirements for growth given by the Minnesota Standard published by Eckles and Gullickson (5). The total calcium and inorganic phosphorus content of the blood plasma were determined from successive 3-day composite samples at least once every thirty days.

Standard methods were followed in conducting the balance trials. In order to accommodate steers in the metabolism stall special equipment was designed,[2] as shown in Plate VI. A heavy rubber funnel was made from a large automobile inner tube. The top was clamped between two tape-covered metal rings which were then bolted together and bent somewhat to conform to the contour of the animal's body. The funnel opening was held in place over the opening of the animal's sheath by straps around the body. A small-bore, heavy-walled rubber tubing connected to a stopcock cemented into the lower end of the funnel, served to conduct the urine to the collecting pan. The calcium and phosphorus analyses of the excreta and feedstuffs were carried out by the methods of Morris, Nelson, and Palmer (6).

Vitamin-D assays using rats were made on the two lots of prairie hay and the beet pulp used in the calf-feeding work. The method followed was that given in U. S. P. X Interim revision for the assay of cod liver oil for vitamin-D.

OBSERVATIONS

The calcium and phosphorus retention of normal calves.—Three Holstein calves, E-180, E-183 and 436 and one Jersy calf, E-181 were used in this work. The ration included prairie hay and a concentrate mixture composed of the common grains and grain by-products. The calves had been exposed to sunshine during the summer and otherwise cared for in the customary manner. They appeared normal and showed a normal concentration of calcium and inorganic phosphorus in the blood plasma. During the balance trials the animals were exposed to sunshine on favourable days. As shown in Table 1, these calves, seven to nine months of age, retained an average of 4·17 grms. of calcium and 2·61 grms. of phosphorus daily.

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[2] Credit is due to Mr. M. E. Mattison, Curator of the Biochemistry Division for assisting in designing and making this device.

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PLATE VI
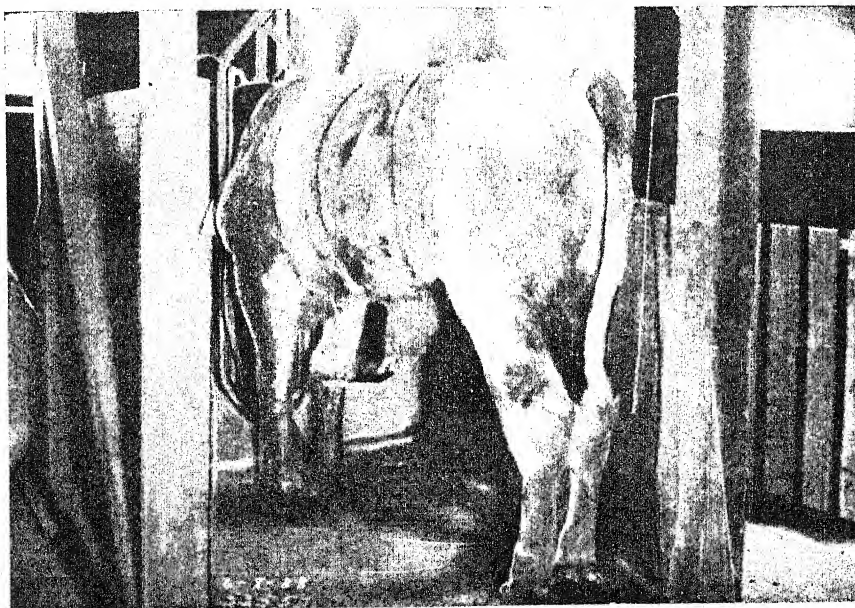
E—201 in metabolism stall showing special equipment designed for collecting urine in balance trials.

TABLE I

The results of mineral balance trials showing the calcium and phosphorus retention of four normal calves

Animal No.	Age at beginning	Weight	Total sunshine	Blood Plasma		Mineral Balance (10-day Period)					
				Total Ca	Inorganic P	Ca			P		
						Intake	Outgo	Balance	Intake	Outgo	Balance
	Days	lbs.	hrs.	Mgm. per 100 cc.	Mgm. per 100 cc.	gms.	gms.	gms.	gms.	gms.	gms.
E-180 .	290	479	0	9·91	7·46	105·4	53·8	+54·6	77·8	44·9	+32·9
E-181 .	288	397	0	10·41	7·82	95·6	64·4	+31·2	69·4	47·9	+21·5
E-183 .	220	415	2	10·48	7·93	119·1	59·4	+59·7	119·6	91·1	+28·5
436 .	269	465	2	10·15	7·17	132·5	110·9	+21·6	135·1	113·4	+21·7
Average. (10 days).	...	...	...	...	...	...	...	+41·7	...	...	+26·1
Daily average	...	...	...	...	...	...	...	+4·17	...	...	+2·61

Experiments using hay as roughage.—As soon as the balance trials under normal conditions had been completed, these calves and E-182, a grade Holstein, were started on their respective experimental schedules.   All the animals were given an allowance of prairie hay which represented about the amount they would consume *ad libitum*.   E-180, E-181 and E-182 ran parallel on a basal ration adjusted to furnish 30 grms. of calcium and 15 grms. of phosphorus daily per 1,000 pounds of live weight.   E-180 was kept indoors, E-181 was allowed sunshine exposure on favourable days, E-182 was kept indoors but given a vitamin-D supplement of 2 cc. of viosterol (250 D) daily.   E-183 and 436 were paired on a similar basal ration but with the phosphorus intake increased to 30 grms. daily per 1,000 pounds of live weight.   E-183 was kept indoors while 436 received sunshine exposure.

After about three months on the experimental programme, a series of balance trials were run to note any possible effect of the various procedures on the retention of calcium and phosphorus although none of the animals were showing manifestations of a vitamin-D deficiency.   The results of these trials given in Table II show essentially normal retentions of calcium and phosphorus for all the calves. The viosterol supplement of E-182 had not increased her mineral retention above that of E-180, neither had the sunshine exposure of 436 made possible larger retentions for this animal than for E-183 likewise the increased phosphorus intake of E-183 and 436 had not brought about an increased retention of this element as compared with that of E-180 and E-182.   As all of these animals continued through the winter in essentially normal condition no further balance trials were conducted on this group.

TABLE II

The results of mineral balance trials showing the calcium and phosphorus retention of calves receiving prairie hay as roughage with and without mineral and vitamin-D supplements

Animal	Age at beginning	Weight	Supplements Mineral	Supplements Vitamin-D	Blood Plasma Total Ca	Blood Plasma Inorganic P	Mineral Balance (10-day period) Ca Intake	Ca Outgo	Ca Balance	P Intake	P Outgo	P Balance
	Days	lbs.			mgm. per 100 cc.	mgm. per 100 cc.	gms.	gms.	gms.	gms.	gms.	gms.
E-180	431	670	None	None	10·84	5·85	210·3	133·3	+77·0	96·4	57·1	+39·3
E-182	394	677	None	Viosterol[1]	10·68	7·09	210·7	140·0	+70·7	96·4	53·2	+43·2
E-183	318	580	Phosphorus	None	9·51	8·03	179·8	68·6	+111·2	174·0	128·6	+45·4
436	364	622	Phosphorus	Sunshine[2]	10·46	7·72	190·3	98·9	+91·4	182·1	150·2	+31·9
E-169	610	871	None	Viosterol	9·98	8·13	113·5	48·2	+65·3	113·3	78·0	+35·3
E-169	680	935	None	None[3]	9·61	7·30	132·7	57·9	+74·8	117·8	79·9	+37·9
E-170	592	728	Calcium	None	9·45	5·85	573·6	498·7	+74·9	91·0	66·3	+24·7
E-170	662	787	Calcium	Viosterol[4]	10·62	4·98	664·7	626·8	+37·9	96·5	72·5	+24·0
Average (10 days)	...	...	...	...	...	...	...	...	+75·4	...	...	+35·2
Daily average	...	...	...	...	...	...	...	...	+7·54	...	...	+3·52

[1] Two cc. viosterol daily during entire experiment. [2] Sunshine exposure during entire experiment. [3] Viosterol removed 17 days previous to this trial.
[4] Five cc. viosterol daily added 17 days previous to this trial.

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The results obtained with E-169 and E-170, grade Holstein females, are also given in Table II. These animals were available from another experiment in which they had developed a rachitic syndrome on a low-calcium-low-phosphorus ration under indoor conditions. E-169 had been growing vigorously throughout the summer after receiving a viosterol supplement with no further change in her experimental schedule. E-170 had shown slow but persistent improvement after the addition of $CaCO_3$ to her ration. Both animals were receiving prairie hay. The results from the first balance trial with each animal indicate that the viosterol was enabling E-169 to retain adequate amounts of calcium and phosphorus from her limited intake, and that E-170 was retaining adequate amounts by virtue of the increased calcium intake without additional vitamin-D. The viosterol was now removed from the ration of E-169 and added to that of E-170 at the rate of 5 cc. daily. After seventeen days, balance trials were again run. E-169 was still retaining approximately the same amount of calcium and phosphorus. The added viosterol had not improved the mineral retention of E-170, in fact, it was somewhat less but still within the limits of variations reported for normal animals.

The results of the balance trials run on this group of calves receiving hay as roughage are essentially normal in all cases. None of the calves showed the characteristic symptoms of a vitamin-D deficiency. As E-180 and E-183 were on the basal schedule without additional vitamin-D for six to seven months it would seem that a reasonable length of time had elapsed in which to deplete the possible vitamin-D stores of a young calf at weaning time, hence it seemed more likely that all the animals were getting this factor from some other source. In view of the fact that several investigators have found a varying, yet appreciable, amount of vitamin-D in different kinds of hay, it seemed that the prairie hay was the most likely source of an appreciable amount of this factor in the experimental schedule of the control calves on the basal ration. To check this point with approved methods, the prairie hays and beet pulp used as roughage were subjected to vitamin-D assays by use of laboratory animals. Both lots of prairie hay were found to carry appreciable amounts of vitamin-D which affords a logical explanation for the essentially normal condition and normal mineral retentions of the calves on the basal ration and the lack of any appreciable differences in the retentions of those getting varying amounts of vitamin-D supplements from different sources.

The vitamin-D assays with laboratory animals were designed to be qualitative rather than quantitative. Preliminary tests indicated that rats would readily consume the test diets containing 45 per cent of powdered prairie hay and 25 per cent of beet pulp which corresponded to the levels of these materials in the calf rations. The calcium/phosphorus ratio of the rachitogenic diet was changed but slightly when the test materials were added, the tendency being for a wider ratio.

The results of the line test assays carried out according to the requirements of the U. S. P. X Interim revision of the assay of cod liver oil for vitamin-D are shown in Table III.

TABLE III

The result of Vitamin-D assays by laboratory animals, showing the food intake and the degree of healing for the various test materials

Test Material	Amount in Diet	Number of Rats	Average daily food intake			Average Degree of Healing
			Rachitogenic Period	Assay period		
				Test diet	Latent Rachitogenic diet	
	Per cent		gms.	gms.	gms.	
Prairie Hay—Lot 45 . .	45	12	6·6	9·8	8·4	1·46
Prairie Hay—Lot 45 . .	30	4	5·9	8·3	7·1	0·75
Prairie Hay—Lot 45 . .	15	4	5·9	7·8	7·2	0·00
Prairie Hay—Lot 55 . .	45	13	6·0	8·5	8·5	2·38
Beet Pulp—Lot 7 . .	25	5	5·3	6·0	6·7	0·00
Rickets Diet—21 day controls ·	...	2	5·9	...	...	Wide Metaphyses.
Rickets Diet—31 day controls .	...	2	6·3	...	6·5	Wide Metaphyses .

* The line tests were assigned values of 0, +, ++, etc., and the numerical value of pluse averaged to give the data in this column.

Experiments using beet pulp as roughage.—The animals used in this experiment were E-201, a grade Shorthorn steer about one year old, received from a farm in a vitamin-D deficient condition ; E-185, a grade Guernsey female thirteen months old ; and E-194, a purebred Holstein, eleven months old.

After being received from the farm, E-201 was maintained for about six weeks under indoor conditions on a diet similar to that received on the farm. This included two pounds of prairie hay daily and a grain mixture composed of corn, oats, and corn gluten meal. The calf was then in excellent experimental condition for determining the calcium and phosphorus retention of a growing animal suffering from a vitamin-D deficiency. The deficiency was evidenced by his rachitic-like condition and the low concentration of calcium and inorganic phosphorus in the blood plasma as noted in Table IV, the first balance trial for E-201. After this trial the ration remained the same except for the addition of 5 cc. of viosterol (250 D) daily. The response to this treatment was prompt and marked. The blood picture was normal within three weeks, the stiffness had largely disappeared, the animal was more alert and active, and the appetite was improving. At the end of four weeks he was placed on a balance trial again to ascertain the nature of the calcium and phosphorus retention on the same mineral

level but with the animal rapidly approaching a normal condition following the vitamin-D therapy. The improvement in calcium retention from 1·44 grms. to 6·82 grms. daily was nearly five-fold, while the increase in phosphorus retention from 1·10 grms. to 2·68 grms. daily was slightly less than three fold.

Shortly after the completion of this second trial observations indicated that the animal was apparently normal, whereupon the viosterol was removed from the ration, the experimental schedule otherwise being continued as before. There was a steady decline in the blood plasma calcium from 10·15 to 6·12 mgm. per 100 cc. and in inorganic phosphorus from 8·26 to 5·05 mgm., during the next three and one-half months, at which time the other rachitic-like symptoms began to appear. These observations indicated that body stores of vitamin-D, and/or calcium and phosphorus had been depleted and the animal was again in a vitamin-D deficient condition. At this time a third balance trial was run, followed by a fourth about five weeks after the ration had been again supplemented with 5 cc. of viosterol. As noted in Table IV, the increase in calcium and phosphorus retention was even more striking than before and serves to indicate the important role played by vitamin-D in promoting the mineral retention of vitamin-D deficient calves.

TABLE IV

The results of mineral balance trials showing the calcium and phosphorus retention of calves, receiving beet pulp as roughage, with and without mineral and vitamin-D supplements

Animal	Age at Beginning	Weight	Supplements		Blood Plasma		Mineral Balance (10-Day Period)					
			Mineral	Vitamin-D	Total Ca	Inorganic P	Ca			P		
							Intake	Outgo	Balance	Intake	Outgo	Balance
	days	lbs.			mgm. per 100 c.c.	mgm. per 100 c.c.	gms.	gms.	gms.	gms.	gms.	gms.
E-201	...	527	None	None	6·42	3·56	71·7	57·2	+14·5	83·8	72·8	+11·0
E-201	...	539	None	Viosterol[1]	10·09	6·87	96·4	28·2	+68·2	83·8	57·0	+26·8
E-201	...	775	None	None[2]	6·12	5·05	85·8	70·7	+6·1	122·2	116·5	+5·7
E-201	...	786	None	Viosterol[3]	9·29	7·94	102·9	50·1	+52·8	119·8	96·4	+23·4
E-185	475	485	CaCO₃	None	8·41	3·13	271·2	292·0	−20·8	38·5	48·8	−10·3
E-185	538	461	CaCO₃	Viosterol[4]	11·15	4·98	240·4	184·0	+56·4	38·9	21·5	+17·4
E-194	430	575	CaCO₃	None	8·20	3·40	356·2	335·5	+20·7	48·8	45·6	+3·2
E-194	462	625	CaCO₃	Viosterol[5]	11·11	6·41	456·0	333·0	+122·4	72·1	32·3	+30·8

[1] Started feeding 5 cc. of viosterol daily 33 days previous to this trial.
[2] Viosterol feeding discontinued approximately 3½ months previous to this trial.
[3] Started feeding 5 cc. of viosterol daily 40 days previous to this trial.
[4] Started feeding 5 cc. of viosterol daily 53 days previous to this trial.
[5] Started feeding 5 cc. of viosterol daily 23 days previous to this trial.

It is conceivable that the level of min eral intake may influence the effect of vitamin-D on mineral retention. E-185 and E-194 developed a severe rachitic-like condition on a ration of beet pulp and grain mixture furnishing a very limited calcium intake. A slight temporary improvement in the blood plasma calcium resulted when a $CaCO_3$ supplement was added to bring the intake of E-185 to 40 grms. daily and E-194 to 50 grms. daily. By the end of a month it was declining again and the physical condition was very poor. E-185 was showing considerable anorexia, and E-194 was also slightly off feed so that the mineral intake indicated in the balance trials run at this time is somewhat lower than the prescribed amounts previously consumed. The results are shown in Table IV as the first balance trial for each animal, respectively. The inability of vitamin-D deficient calves to make normal mineral retention on rations supplying an abundance of calcium and normal phosphorus is indicated by the fact that E-185 was in negative calcium and phosphorus balance while E-194 was slightly better than in equilibrium.

A second balance trial was run on each animal after the ration had been supplemented for some time with viosterol. Under the influence of the added vitamin-D both animals now showed very satisfactory positive balances of both calcium and phosphorus, as may be noted in the second trial for each animal as recorded in Table IV.

Although the appetites were now good, the nutrient and mineral intake of E-185 was held down to the same amount that she consumed on the previous trial, but for unavoidable reasons the mineral intake, especially the calcium, was somewhat larger for E-194 in the second trial than in the first. However, the calcium intake of E-194 was liberal, and more than sufficient in both trials so that the variation undoubtedly does not introduce any appreciable difficulty in interpreting the results. The increase in the calcium intake was approximately 100 grms. for the 10-day period, while the increase in retention was about 102 grms. The tendency is for the percentage of mineral retention to decrease with an increase in the intake, but even by assuming that the retention of the extra 100 grms. of calcium in the second trial would have been at the same rate as in the first trial there would have been an increase of only about 5·81 grms. in the second trial without the added vitamin-D. The fact that over 100 grms. of extra calcium were retained in the second trial indicates clearly the beneficial effect of the added vitamin-D.

The results secured with E-185 and E-194 substantiated the observations already made on E-201 and indicate that a liberal calcium intake will not suffice to promote adequate mineral retention in calves suffering from a vitamin-D deficiency.

The relation of vitamin-D to aphosphorosis.—Two grade Holstein females, E-187 and E-190 twelve and ten months of age, respectively, were used in an attempt to study, the effect of vitamin-D on the mineral retention of calves suffering from aphosphorosis. The basal ration furnished liberal calcium but a restricted amount of phosphorous, and was typical of those upon which aphosphorosis develops except that vitamin-D-free beet pulp was substituted for the usual hay roughage and the calves were kept indoors. By the end of two months it began to appear that uncomplicated aphosphorosis could be obtained only in the presence of at least some vitamin-D. The blood plasma calcium as well as the inorganic phosphorus began to decline and the physical condition more nearly resembled that of the rachitic syndrome than simple aphosphorosis. There was no evidence of pica. The onset of physical disturbances was rather sudden and severe with E-190 so that she had to be dropped from the experiment. A balance trial was run on E-187 although her condition was not that of simple aphosphorosis. The results of this trial on the basal ration are shown in Table V. The mineral retention is about what might be expected for a mildly rachitic animal.

TABLE V

The results of mineral balance trials on E-187 showing the calcium and phosphorus retention on the basal ration,[1] basal ration plus phosphorus, and basal ration plus viosterol.

Type of Ration	Age at Beginning	Weight of Animal	Blood Plasma		Mineral Balance					
			Total Ca	Inorganic P	Ca			P (10-day period)		
					Intake	Outgo	Balance	Intake	Outgo	Balance
	days	lbs	mgm. per 100 cc.	mgm. per 100 c.c.	gms.	gms.	gms.	gms.	gms.	gms.
Basal	478	694	8·66	3·45	294·5	237·2	+57·3	77·1	53·5	+23·6
Basal *plus* P[1]	501	700	6·12	5·26	103·9	127·6	—23·7	41·1	63·2	—22·1
Basal *plus* viosterol[3]	574	630	10·73	7·73	270·5	192·2	+78·3	80·7	34·6	+46·1

[1] Basal ration furnished liberal Ca, restricted P, typical of those producing aphosphorosis except that beet pulp was substituted for the customary hay roughage, and animal was kept indoors. Alterations resulted in development of rachitic syndrome rather than uncomplicated aphosphorosis.

[2] $NaH_2PO_4.H_2O$ added to increase P intake to 18 gms. daily 7 days previous to this trial. Animal badly off feed during this trial.

[3] Animal received viosterol for 2 months previous to this trial.

A second trial was run about a week after a supplement of monobasic sodium phosphate had been added to her ration, to study the effect of added phosphorus on the calcium and phosphorus retention of vitamin-D deficient animals and to learn the nature of the mineral retention of such animals receiving a liberal intake of both calcium and phosphorus. The animal became worse rapidly and went off feed so badly that a satisfactory balance trial to indicate the effect of added phosphorus was not obtained. The results of the trial are shown in Table V. The fact that a severe breakdown seemed to be hastened would indicate that the effect undoubtedly was not very favourable.

The phosphorus supplement was now removed from her ration and viosterol added. As the animal was nearing recovery a third balance trial was run with the ration adjusted to duplicate that of the first trial except for the added viosterol. The results given in Table V show a considerable improvement in both the calcium and phosphorus retention over that obtained when the animal was mildly deficient in vitamin-D and thus substantiate the previous observations as to the favourable effect of vitamin-D.

DISCUSSION

The favourable effect of vitamin-D on the calcium and phosphorus retention of calves suffering from a vitamin-D deficiency is strikingly shown by the results obtained with E-201, E-185, and E-194. The vitamin-D deficiency of these animals was indicated by sub-normal concentrations of calcium and inorganic phosphorus in the blood plasma, stiffness, bending of the knees, swelling of the knee, hock and pastern joints, humping of the back, and often some inanition. As shown in Table VI, the average daily retention of these calves at an age when bone mineralization should have been progressing rapidly was only 0·51 grms. of calcium and 0·24 grms. of phosphorus. When viosterol was added to supply vitamin-D, and without any other appreciable change in the ration, there was a marked increase in the calcium and phosphorus retention in every case, the daily average now being 7·49 grms. of calcium and 2·68 grms. of phosphorus. These amounts represent essentially normal retentions. The increase brought about by the vitamin-D administration in from three to seven weeks was approximately fourteen-fold for calcium and eleven-fold for phosphorus. Coincident with the marked improvement in the mineral retention there was a prompt return of the calcium and inorganic phosphorus of the blood plasma to normal concentrations, and a corresponding improvement in the physical well-being of the calf. With such decided and consistent results there can be no doubt of the important role played by vitamin-D in promoting the retention and utilization of calcium and phosphorus by calves under these conditions.

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TABLE VI

The calcium and phosphorus retention of calves before and after vitamin-D administration

Animal Number			Mineral Balance (Ten-Day Period)			
			Before feeding viosterol		After feeding viosterol	
			Ca	P	Ca	P
			gms.	gms.	gms.	gms.
E-201 .			+14·4	+11·0	+68·2	+26·8
E-201 .			+6·1	+5·7	+52·8	+23·4
E-185 .			—20·8	—10·3	+56·4	+17·4
E-194 .			+20·7	+3·2	+122·4	+39·8
Average (10-day period)			+5·1	+2·4	+74·9	+26·8
Daily average			+0·51	+0·24	+7·49	+2·68

Increasing the mineral content of the ration of vitamin-D deficient calves had no such favourable influence on the mineral retention. Increasing the calcium intake of E-185 and E-194 to 40—50 grms. daily failed to promote adequate mineral retention, in fact, E-185 was in negative balance. Although the evidence for the effect of added phosphorus in the case of E-187 is not as conclusive, the fact that adding a liberal amount of phosphorus to the ration of this animal suffering from a mild vitamin-D deficiency did not prevent, and may even have hastened, a severe breakdown so that a satisfactory balance trial could not be obtained would cast considerable doubt on the possibility of any beneficial effects of the added phosphorus.

Further information on the mineral retention of normal growing calves is also afforded. Data for the mineral retention of four normal calves, seven to ten months of age, have been given in Table I. As the calves in the experimental group receiving prairie hay as roughage completed the experiment in essentially normal condition, and the rations were similar to those commonly fed in practice, it would seem that these results might also be considered as representative of essentially normal retentions. The data for this group have been given in Table II. When the results of the above two groups are averaged together, the grand average of all the balances with essentially normal calves shows an average daily retention of 6·42 grms. of calcium and 3·22 grms. of phosphorus. These results corroborate the work of Lindsey, Archibald, and Nelson (7), who obtained normal retentions of 7·74 grms. of calcium and 3·52 grms. of phosphorus daily for a high calcium group and 4·45 grms. of calcium and 2·15 grms. of phosphorus daily for a low calcium group.

The average normal daily retention of 6·42 grms. of calcium and 3·22 grms. of phosphorus obtained in this work is almost exactly in a two to one ratio irrespective of the varying amounts in the ration fed.   This closely approximates the ratio of these two elements in the bodies of growing and mature cattle.   Lindsey, Archibald, and Nelson (7) noted the same relationship in their work with normal animals.   It is of interest to note that in this work the slight average daily positive balances of 0·51 grms. of calcium and 0·24 grms. of phosphorus shown by the rachitic calves are also in an approximately two to one ratio.   Also, that the loss of calcium and phosphorus in the case of E-185 showed this ratio.   Such observations indicate an ultimate interdependence between the two elements and suggest that a deficiency in one might be responsible for a lack of retention of the other.

The fact that the calves receiving prairie hay as roughage continued throughout the experiment in an apparently normal condition irrespective of whether or not they received any vitamin-D supplement, whereas those on a similar or even higher mineral intake but receiving beet pulp soon showed symptoms of a vitamin-D deficiency, indicates that the prairie hay undoubtedly carried an adequate amount of the antirachitic factor, while the beet pulp contributed very little, if any.   The vitamin-D assays with laboratory animals furnished corroborative and conclusive evidence on the above points.   When the two lots of prairie hay were fed to rachitic rats definite healing was obtained, although it was more pronounced for one lot than for the other.   No signs of healing were evidenced when the beet pulp was fed.

The assays furnish a basis for estimating the amount of vitamin D supplied the calves by the hay contained in their rations.   Rough calculations indicate that the average hay allowance supplied the calves with approximately 135 Steenbock units of vitamin-D daily, which served to protect the calves from developing the rachitic-like syndrome on the level of mineral intake furnished by these rations. Just how much less may have sufficed to protect the animals cannot be determined from these observations.   These estimation indicate that the vitamin-D requirement of this species is relatively small as compared with the requirement of infants as recently reported by Hess and Lewis (8), while the fact that an increased mineral intake and changes in the calcium/phosphorus ratio failed to protect the calves or initiate appreciable healing suggests the possibility that calves are less independent of vitamin-D than the rat.

Some suggestions as to the amount and length of storage of vitamin-D by calves are also indicated by the results obtained. The viosterol fed to the calves as a therapeutic agent supplied roughly 15,000 Steenbock units daily, which is over 100 times the amount furnished by the hay. The exact amount which would have proved sufficient is not known but if the vitamin-D in the viosterol had been used efficiently and the excess stored, the animals should have accumulated

sufficient for protection for a comparatively long period of time. When the viosterol was removed from the ration of E-201 after the second balance trial the previous stores of vitamin-D made during two and one-half months of viosterol feeding were depleted in about three and one-half months, as evidenced by his physical condition and the low concentration of calcium and inorganic phosphorus in the blood plasma. The time interval would undoubtedly have been shortened had the two pounds of prairie hay allowed part of the time been replaced with beet pulp all of the time. These observations suggest several possibilities. For instance, the Vitamin-D in irradiated ergosterol may not be the most effective for this species so that the excess available for storage was small, or it may be inefficiently absorbed, or if absorbed, it may be re-excreted. It is also possible that the calf has only limited ability for storing vitamin-D at best. The observations on these points are very meagre and the possibilities mentioned are made merely as suggestions. The results do indicate, however, that a young growing calf may make a storage of vitamin-D under favourable circumstances which may be used as a protection against a deficiency of this factor for a varying length of time under adverse conditions.

CONCLUSIONS

The following conclusions may be drawn from the results of this investigation.

1. The calcium and phosphorus retention of vitamin-D deficient calves is very markedly improved by the administration of vitamin-D. The average calcium retention may be increased fourteen-fold and the phosphorus retention eleven-fold by vitamin-D therapy.

2. Increasing the mineral content of the ration of vitamin-D deficient calves has no favourable influence on the mineral retention.

3. Based on the limited evidence obtained, the average daily retention of normal calves approximated $6 \cdot 50$ grms. of calcium and $3 \cdot 25$ grms. of phosphorus.

4. Judging from the results of this experiment, calcium and phosphorus are retained by normal calves in approximately a two to one ratio regardless of variations in the mineral content of the ration. The statement is equally true for the small average daily retentions made by calves suffering from a vitamin-D deficiency. These observations indicate an ultimate inter-relationship between these two elements and suggest that a shortage of either one in the ration might act as a limiting factor in the retention of the other.

5. Prairie hay may carry appreciable amounts of vitamin-D. Beet pulp, on the other hand, probably possesses very little, if any, anti-rachitic potency.

6. A young growing calf may store vitamin-D under favourable conditions to be used as a protection against a deficiency of this factor for a varying length of time under adverse conditions.

7. Uncomplicated aphosphorosis does not develop when all sources of appreciable amounts of vitamin-D are eliminated from rations otherwise similar to those which ordinarily bring about this condition.

8. Vitamin-D acts to improve the mineral retention of calves suffering from a rachitic-like syndrome within at least three to seven weeks after its administration.

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BALANCED DIETS, NET ENERGY VALUES AND SPECIFIC DYNAMIC EFFECTS

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A fact that is not infrequently lost sight of in contemporary nutritional research [1] is that the utilization of any food nutrient for any purpose in the animal body requires the simultaneous presence of all other nutrients required for that purpose. And for the most complete sustained utilization of any food nutrient, the proportions in the diet of it and all other required nutrients must attain or exceed certain minimum values. For example, an adult man may require forty grms. of protein daily, although, consuming only this, he will not be able to establish nitrogen equilibrium. If his energy requirements are simultaneously covered, he may be able to establish nitrogen equilibrium, but there is no reason to expect that he could prevent losses of nitrogen from his body indefinitely unless his daily diet contains also at least certain minimum proportions of each nutrient, inorganic as well as organic, that is required for all the animal functions essential to the maintenance of life. He is then receiving what may be called a " balanced diet " for adult maintenance. It is of course well known that diets may be unbalanced by including in them excessive proportions of some nutrients, such as protein or vitamin D, but this is a phase of the problem about which little definitely can be said.

For the growing animal we have a similar conception of a balanced diet, and in this case there is available much more information concerning nutrient requirements, for in the science of nutrition, as in the medical sciences, the adolescent animal has received much more attention than has the adult.

It is reasonable to assume that the balanced or unbalanced character of a diet for growth will be reflected in the efficiency with which that diet promotes growth. The completely balanced diet will promote growth the most efficiently, in the sense that, when compared with any less completely balanced diet in properly controlled feeding experiments, a greater rate of growth will be secured on the same amount of food. This is the principle underlying the Armsby " paired feeding " method, the most precise method that has yet been proposed for effecting ration comparisons. In this method, animals are paired on the basis of sex, parentage, weight and

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[1] For example, vitamin units are commonly defined as amounts that will produce certain more or less well defined physiological effects. In these definitions no reference is made to the simultaneous necessity of other nutritive factors, and in the methods used for vitamin assay no provision is made to assure adequate, or even constant, intakes of other nutrients.

any other measurement (such as blood hemoglobin) that may be pertinent to the problem at hand, and the pair mates are then fed the same amount of the two rations to be compared, one to one animal and one to the other. Under these conditions the better balanced of the two rations will promote the more rapid growth, or in other ways induce a better nutritive condition, for example with reference to the blood or the bones ; and conversely every improvement in a diet with respect to its power to promote growth and nutritive condition is *prima facie* evidence of a betterment in its balance. The reality of this conclusion seems obvious, and no precise definition of balance in diet on any other basis has been proposed in so far as the writer is aware. In fact, a search of current writings and text-books has failed to reveal that the conception of nutritive balance in diets or rations has received much intensive thought. On the evidential side, the conclusion is supported by the results of numerous paired-feeding experiments, which have demonstrated the possibility of distinguishing on this basis better from poorer balanced diets with respect to protein, vitamins, sugars and inorganic salts.

Of the balanced ration, it may be said that the more of it is consumed, the better nourished will be the animal with reference to which the ration is balanced, up to the point of repletion of its requirements. It is an attractive hypothesis concerning unbalanced rations that the more of them are consumed the poorer nourished will be the animal with reference to the functions with respect to which the rations are unbalanced. To the writer the hypothesis has much rational appeal and it receives factual support from some experiments performed in the nutrition laboratory at the University of Illinois. Thus, young growing rats subsisting on a diet of milk will develop anemia, because milk is unbalanced with respect to the requirements of the hematopoietic tissues ; and furthermore the more milk the animals consume daily, the more rapidly will the anemic condition develop, although in all other respects the animals are well nourished. Again, young rats placed upon a diet high in calcium, low in phosphorus and deficient in vitamin D will develop rickets, and the rate of development of this bone disease is the greater, the greater the daily consumption of the rachitogenic diet. Of much the same significance is the fact that young pigs placed upon a protein-deficient diet will grow slowly, but with increasng intakes of food will become increasingly fat, representing a misdirected or uncoordinated growth. Probably further illustrations of the hypothesis that unbalanced rations, like toxic substances, exert harmful effects in proportion to the amounts consumed, will be forthcoming when it is subjected to systematic and quantitative study. The quite general failure of animals to consume unbalanced rations as avidly as balanced rations is understandable if the former may be considered physiologically harmful.

Some of the implications of the above conceptions of nutritive balance in diet are interesting and of importance to the science of nutrition. Attention will be restricted to the question of the utilization of the chemical energy contained in a diet, representing a nutritive summation of all the organic nutrients.

There is current in animal nutrition a method of assessing the energy value of rations that is far in advance of any method used in human nutrition. According to this method, introduced by Armsby some thirty years ago, the final value of a ration as a source of energy in metabolism is obtained by deducting from its gross energy (heat of combustion) all the losses of energy incident to its utilization. The metabolizable energy is the gross energy minus indigestible energy (gross energy of faeces and intestinal gases) and unoxidized energy (gross energy of urine). The final, or net energy value is equal to the metabolizable energy minus the increase in the heat production incident to the consumption and utilization of the ration. This latter increment consists largely (in farm animals) or entirely (in humans) of the " specific dynamic effect " of food. The net energy of a unit weight of a ration, expressed as a percentage of the contained metabolizable energy, measures the net availability of the latter.

With these definitions in mind, the first implication of the above defined conception of nutritive balance in a ration or diet is that, except for differences in digestibility, the net energy value of all perfectly balanced rations is the same under the same conditions of feeding, or, somewhat more precisely, the net availability of the metabolizable energy of all perfectly balanced rations is maximal for any imposed conditions of feeding. When the net energy conception was developed by Armsby, it was his idea that each food material had its own fixed characteristic net energy value and that the net energy value of a ration was the weighted mean of the net energy values of the constituent foods. All his investigations at Pennsylvania State College were based upon this simple hypothesis, which was not inconsistent with any of the theories of energy utilization prevalent at that time. Forbes and his associates have been impelled to depart from this hypothesis of Armsby, first, because of experimental evidence to the effect that the net energy value of a ration or feed is not constant, but depends upon the conditions of feeding and second, because other evidence indicated that the net energy value of a ration bore no simple relation to the net energy value of the costituent foods. However, Forbes' recently announced "law of maximum normal nutritive value,"[2] although it advocates the use of completely balanced rations in determinations of net energy values, does not state nor imply that the net availability of the metabolizable energy of such rations will be maximal and identical. In fact, the statement that " an individual foodstuff expresses its normal and most characteristic nutritive value ...... only as it is a part of a ration which is qualitatively complete and quantitatively sufficient .. " seems opposed to this deduction, which, if true, would lead one to suppose that the most characteristic nutritive value of a food would be observed only when it is fed alone. When properly balanced with other foods, its distinctive nutritive properties would be entirely submerged in a resultant optimal combination that would be no better nor worse than that of many other

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[2] *Science.* 77 : 306, 1933.

possible mixtures of foods.   The recent developments in the net energy conception,
initiated and defended by the Pennsylvania group have tended to complicate the
problem of net energy determinations and perhaps even to discourage those who
have hoped to put the conception to practical use in the rationing of farm animals.
But if all perfectly balanced rations exhibit the same net energy value (except for
differences in digestibility) under the same conditions of feeding, then the problem
is greatly simplified and the plan of its solution is clear ; furthermore, the proba-
bility that the solution will be sufficiently simple to possess great practical value
is enhanced.

A second important implication from the conception of a balanced diet
developed above is that the specific dynamic effects of the various nutrients are
not characteristic values except when they are fed to animals singly.   When the
nutrients are fed in combinations, the specific dynamic effects of the mixtures will
be less than the weighted mean of the individual effects, and this decrease will
continue as the combinations approach a perfectly balanced combination for the
animal under experiment, of which the heating effect will be minimal.   In this
discussion, the term " specific dynamic effect " will be applied to the total excess
heat developed by a given food or nutrient, and not the so-called " peak "
effect, which possesses an extremely limited significance.

This is a decidedly heretical deduction. It is, however, a direct corollary of the
preceding implication, since if the net availabilities of the metabolizable energy
of all perfectly balanced rations are maximal and identical, then their specific
dynamic effects must be minimal and identical.

Current theories attach definite heating effects to proteins, sugars, starches,
fats, and even the various naturally occurring amino-acids, and teach that com-
bination of these nutrients does not modify appreciably their characteristic effects
as metabolic stimulants, but these theories fail to account for many facts in the
science of nutrition.   Eight years ago Carman and the author[3] showed that the
mere inclusion of 1 per cent of sodium chloride in a ration predominantly made up
of corn increased its growth-promoting value by from 40 to 50 per cent in paired-
feeding experiments with rats and chicks, without appreciably affecting its digesti-
bility.   Assuming reasonably that this effect could not have been the result of a
depression of the basal metabolism or of the muscular activity of the experimental
animals, it must have been an expression of a great increase in the net energy value
of the ration and as great a depression in its specific dynamic effect.   As we said at
the time : "The growth data of this experiment afford a striking illustration of the
fact that the utilization of food energy by growing animals may be greatly impaired
by an improper balance among indispensable dietary factors".   None of the
current theories of the cause of the specific dynamic effect of food would seemingly
account for this phenomenon.

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[3] *Jour. Biol. Chem.*, **68** : 165, 1926.   M

Protein ingested   alone by animals causes a marked specific dynamic effect, much greater than any other nutrient, but when incorporated into a protein-free diet, otherwise complete, it must decrease the specific dynamic effect of such a diet rather than increase it, because the combination will be more efficient in maintaining the energy balance of an adult animal or in increasing the energy balance of a growing animal. Weiss and Rapport [4] were greatly mystified when they found that calorigenic amino-acids, administered to dogs along with proteins, failed to increase the calorigenic action of the latter.   But equally mystifying from the standpoint of the current theories of the specific dynamic effects of food is the action of amino-acids in improving greatly the efficiency for growth of rations containing protein complexes deficient in those amino-acids [5].   In all probability this increase in efficiency means a decrease in the specific dynamic effect, assuming again that the basal metabolic rate and the activity of the experimental animals was not depressed by the amino-acid supplements.

Apparently the specific dynamic effects of isolated nutrients fed as such have very little if anything to do with the specific dynamic effects of mixtures of nutrients, particularly balanced mixtures. Without being able to specify the exact causes of the metabolic stimulation induced by the   consumption of food, we may nevertheless conclude reasonably that its intensity is dependent primarily upon the degree of accumulation of the end-products of digestion within the tissues, which is in turn dependent for any given intake of food upon the rate of utilization of these products by the tissues. Their rate of utilization will be determined by the proportions existing among them, such that the better the balance with reference to the requirements of the animal the more rapid the rate of utilization and withdrawal from the cellular fluids. The metabolic stimulation thus occurs only when there is an excess of nutritive material in the tissues, and is to a considerable extent proportional to this excess.   It is possibly a mechanism operating merely for the removal of excess food material from the body cells in the interests of physiological efficiency.

These speculations are now being investigated experimentally in the Division of Animal Nutrition of the University of Illinois.

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[4] *Jour. Biol. Chem.*, **60** : 513, 1924.

[5] H. H. Mitchell and D. B. Smuts, *Jour. Biol. Chem.*, **95** : 263, 1932.

BALANCED DIETS, NET ENERGY VALUES AND SPECIFIC DYNAMIC EFFECTS

BY

E. B. FORBES,

Institute of Animal Nutrition, Pennsylvania State College, U. S. A.

In a recent number of *science*[1], H. H. Mitchell presents a theoretical discussion of the subject of this communication, involving certain of the writer's published conclusions.

After developing a line of argument similar to and in harmony with that of the writer in the publication of the so-designated " law of maximum, normal nutritive value ", Mitchell discusses the significance of this principle in relation to net energy values, saying, in part :

With these definitions in mind, the first implication of the above defined conception of nutritive balance in a ration or diet is that except for differences in digestibility the net energy of all perfectly balanced rations is the same under the same conditions of feeding, or somewhat more precisely, the net availability of the metabolizable energy of all perfectly balanced rations is maximal for any imposed conditions of feeding.

Further he says :

However, Forbes' recently announced " law of maximum normal nutritive value ", although it advocates the use of completely balanced rations in determinations of net energy values, does not state nor imply that the net availability of the metabolizable energy of such rations will be maximal and identical.

It is true that, in my " law of maximum, normal nutritive value " [2], I avoided making any statement or implication to the effect that the net availability of the metabolizable energy of completely balanced rations is maximal and identical (though we had discussed the idea), becasue I cannot conceive of balanced rations —as practicable entities—being so perfectly balanced that there would be no individuality of dynamic effect of the nutrients serving the same purposes in different rations, and that there would be no differences in either the excess nutrients, or in substances present without nutritive value, which would affect the economy of utilization of metabolizable energy.

One must remember, in theorising, that in feeding practice we deal not with pure nutrients, of known identity and character, but—in each feeding stuff—with a vast complication of little-known substances.

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[1] *Science*, 80 : 558—561.
[2] *Science*, 77 : 306—307.

Also, it is only fair to call attention to Mitchell's misstatement to the effect that my law of maximum, normal nutritive value " advocates the use of completely balanced rations in the determination of net energy values ". In publishing this principle I did not mention " completely balanced rations ", but did use the expression " a ration which is qualitatively complete and quantitatively sufficient " —which has a distinctly different meaning in that the idea of a complete diet provides only for the presence of all required nutrients, in the necessary quantities, while the perfectly balanced ration literally— must not only be complete, but must not contain an excess of any nutrient. It is true, however, that, at an earlier date, I had—less carefully—used the expression " completely balanced rations " in a similar discussion [3]. Proceeding further, Mitchell calls attention to my statement that " an individual foodstuff expresses its normal and most characteristic nutritive value—only as it is a part of a ration which is qualitatively complete and quantitatively sufficient .... ". The question which Mitchell raises is, in reality, " which is the normal and most characteristic value of a foodstuff— that determined by its full potentialities, when it is adequately supplemented, or by its limitations, when fed alone ? ". The difference is simply one of point of view. It is normal to use feeding stuffs as components of approximately complete rations ; they are not commonly fed alone ; and I have used the word " characteristic " to mean " representative ".

Mitchell states that " the recent developments in the net energy conception, initiated and defended by the Pennsylvania group, have tended to complicate the problem of net energy determinations and perhaps even to discourage those who have hoped to put the conception to practical use in the rationing of farm animals ".

There have been no recent developments in the net energy conception, so far as I know. It remains as at first proposed, and it is as unassailable as the law of conservation of energy. But there has been much new light cast upon the subject of energy metabolism, and a searching analysis of the problem of determining energy values, in studies published from this institute—which however, should be discouraging only to those who adhere to the objective of determining net energy values *of individual feeding stuffs as constants*.

The idea of determining net energy values of rations, however, is worthy of consideration. This is a logical deduction from the work of this institute. I have made this deduction ; have advocated the determination of such values, and have enumerated some of their apparent uses in the study of problems in the field of animal production [3].

In regard to Mitchell's speculations as to the cause of specific dynamic action, the relation of the dynamic effects of nutrients to the combinations in which they are fed, etc., we do not care to comment, especially since the methods of determination of specific dynamic effects, and the measurements of these effects—in the

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[3] *Proc. Amer. Soc. Animal Production, Ann. Meeting*, 1932 : 32—40.

literature—have been so unsatisfactory, in fact, so largely fallacious, in the light of findings of this institute during the past six years, especially as set forth in a very recent paper by Kriss, Forbes and Miller,[4] which places the problem of determining specific dynamic effects of nutrients in a new and vastly improved position.

The new point of view and procedure depend upon Rubner's idea,[5] [6] of a specific dynamic effect of body substance katabolized, from which follows the hypothesis (Forbes, Braman and Kriss,[6]) of a status of minimum heat production of life in which the energy requirement of the animal would be rendered available without waste of heat—that is, without energy expense of utilization ; heat increments (dynamic effects) as usually determined at planes of nutrition below energy equilibrium being less than the true energy expense of utilization by the amount of the dynamic effect of body nutrients katabolized (Forbes, Braman and Kriss[7]) ; heat increments determined above maintenance, with the heat production of maintenance as the base value, therefore, representing the true energy expense of nutrient utilization.

We are free to admit, however, that if—as we have concluded—net energy values of individual foodstuffs are not constants, because of the supplementing effects of food combination, in rations and other conditions affecting the economy of food utilization then it is conceivable that, for similar reasons specific dynamic effects of individual nutrients likewise are not constants. We have unpublished results on conditions affecting specific dynamic action, and a second year's experiments on the subject are in progress.

The recent studies of this institute on specific dynamic effects and their determination afford an improved basis of understanding and procedure from which to investigate this question. In this connection I would propose that it would save confusion to limit the term " specific dynamic effect " to signify the dynamic effect of specific kinds of nutriment, and to use the equivalent term " heat increment " to signify other dynamic effects—that is, those which are not specific of particular kinds of nutriment.

[4]*Jour. Nutrition*, **8** : 509—534.

[5]" Die Gesetze des Energieverbrauchs bei der Ernahrung ", Leipzig und Wien, 1902, S. 370.

[6]*Jour. Agr. Research*, **37** : 285, 1928.

[7]——————————— **40** : 77, 1930.

ABSTRACTS

La réglementation du trafic frontière [The Regulation of Frontier Traffic.]
PAVLOW, G. (1935). *Office Internat. des Épizoot*, **10**, 303–316.

With the rapid development of the means of communication and the impossibility of any one country existing economically by itself and for itself, the intensive international exchange of animals has come to be a necessity and with this has increased the danger of diffusion of contagious diseases.

With the object of reconciling the two apparently irreconcilable obligations, namely the extension of international commerce in animals and the limitation of epizootics, the different countries have organized frontier veterinary services with a view to controlling the importation and exportation of animals and animal products, but such control measures are frequently envisaged from the standpoint of protectionism and political considerations, the sanitary motives being only secondary.

In the beginning, the measures adopted are confined to a temporary closure of such frontier points as are likely to serve as avenues for the introduction of epizootics. Later, recourse is had to visits, quarantines, certificates of health and place of origin of the animals concerned, and, finally, as *ultima ratio*, to the total closure of the frontier.

A study of epidemiology reveals, however, that certain contagious diseases of animals pass from one country to another across political frontiers, even when the latter are most rigorously guarded. Thus, it has been established by evidence that foot-and-mouth disease, sheep-pox, rabies and swine pest defy the rule of the " closed frontier ", while dourine and glanders are introduced through the agency of the contraband of the frontier, such as horses. On the other hand, sanitary cordons and absolute prohibition along the frontier have been found to prevent the introduction of rinderpest and contagious pleuropneumonia of bovines and horses.

In order to demonstrate the exactitude of the foregoing premises, the author refers to certain observations made in the Balkans and particularly in Bulgaria. [These observations, however, are of sufficient general interest to merit consideration in the present abstract].

While it is difficult to produce actual data to demonstrate the transmission of rabies from one country to another, the author, on *a priori* grounds, stresses the fact of its transmission and expresses the view that it requires for its suppression not merely bilateral but international efforts. In the Balkans, there is no area in the political frontiers which may be regarded as protected in so far as rabies is concerned, the economic structure of the Balkanic countries and the rural tradition inherent in them being eminently favourable for the propagation of the disease.

The appearance of sheep-pox in Bulgaria had been effectively prevented during a period of 5 months in 1934 by the adoption of veterinary police measures and by the abandonment of virus injection (" clavelisation ") *en masse* as a prophylactic measure, as this practice always tended to propagate the disease. By means of two instructive maps, the author illustrates the probable manner of its re-importation on two subsequent occasions during the same year. On the first of these occasions, the foci of infection were wholly disposed along the length of the Danube and on the second, the infection had spread into the interior of the country, the appearance of the disease in

certain villages situated on the Danube having been due to its introduction from Rumania. The packs of sheep taken from one side to the other for grazing on the Danubian islands in consequence of the desiccated condition of the pastures on the two banks, the contraband of sheep transported by boats and the carcases thrown into the river constituted the probable mode of penetration of sheep-pox from Rumania into Bulgaria.

As to foot-and-mouth disease, the author emphasizes the view previously expressed that the appearance of the disease in a Balkanic country is generally followed by its spread along the whole of the peninsula. Specific instances are cited to illustrate this.

Some data are produced to show that, prior to 1924, glanders principally existed in the districts adjoining Dobroudja and Thrace and also amongst the horses of certain Rumanian nomads.

From the available epizootiological data concerning dourine, it would appear that the disease for the first time was encountered in Bulgaria in a stallion imported from Hungary in 1898. The author states that cases of dourine are chiefly noticed in the neighbouring frontier zone of Dobroudja amongst young mares, which constitute the agency through which the disease is imported into Bulgaria.

From the foregoing, one may formulate the two deductions that (1) for prophylaxis against certain epizootics, which especially concern the Balkan Peninsula, an inter-Balkanic arrangement is indicated [similar arrangements may be found desirable in other parts of the world] and (2) the danger of the passage of epizootics exists independently of the activity or of the suppression of international traffic. A case in point was the appearance of sheep-pox and foot-and-mouth disease in the districts of Trn. in spite of the fact that the frontier between Bulgaria and Yugoslavia was entirely closed by means of barbed wire netting.

The author refers to the Franco-Swiss and Franco-Belgian Arrangements and to the Franco-Italian and Franco-German Conventions regulating frontier traffic and the movement of animals for pasturage. The question of frontier protection against epizootics is also frequently dealt with in the Veterinary Commercial Conventions of which it constitutes an integral part, as the Conventions between Rumania and Czechoslovakia [1930], between Yugoslavia and Greece [1927] and between Bulgaria and Turkey [1933].

A comparison of these Arrangements and Conventions shows that, independently of the differences imposed by the conditions peculiar to the contracting countries, they stipulate almost wholly the same rules. Thus, the ententes relative to the pasturage for horses provide for (1) the identification of animals and their brand-marks ; (2) a certificate of health delivered by the mayor testifying that the animals concerned come from a locality free from a given epizootic ; (3) the passage of veterinary authorities across the frontier for the purpose of visiting the animals ; (4) the adoption of combative measures.

The seasonal movements of animals, however, relate to the realm of importation, and in the Convention regulating such traffic the following rules are chiefly stipulated :

(1) the identification of the proprietaries concerned ; (2) the compulsory presentation of a descriptive statement for the identification of beasts of burden or of draught

animals ; (3) a certificate of health and of place of origin ; (4) regular and frequent visits to the animals ; (5) the establishment of a frontier zone, ordinarily of a depth of 15 to 20 kilometres, on both sides of the frontier line ; (6) the obligation of veterinary authorities to communicate mutually, within 24 hours, whenever any disease makes its appearance ; (7) the reciprocation of information concerning the preventive measures applied and the eventual extension of the frontier zone on the appearance of a particularly menacing epizootic. The establishment of a frontier veterinary zone is necessitated by our knowledge of the epizootics, namely, that direct contact is not the only method of propagation of contagious diseases, but that there are other methods to which the frontier line does not always constitute a barrier. It would thus seem desirable to substitute the notion of " frontier zone " for that of " frontier line " and constitute for it a new mode of sanitary control, whilst the feudal spirit which expresses itself in the two opposing manifestations, namely, the authorities of the custom house and contraband, should make room for sanitary collaboration.

The establishment of a frontier veterinary zone alone, however, is not calculated to solve the problem of sanitary protection, and the author stresses the desirability of the unification of veterinary organizations and, particularly, the methods of combating epizootics and suggests the institution of a frontier veterinary service in which each agent will have a special sector in his charge.

While the unification of combative measures against all diseases, without exception, is not possible, it is nevertheless feasible, under a European plan, to unify the zoo-prophylactic measures against rabies, sheep-pox, dourine, rinderpest, rouget, anthrax, blackquarter and cholera of birds.

The author deprecates merely formal collaboration amongst veterinary authorities and refers to the need of creating an intimate liaison amongst them designed to ensure concerted action in the application of combative measures against epizootics. [S. K. S.]

Études sur la pleuro-pneumonic infectieuse des chèvres d'Anatolie (Studies on the infectious pleuro-pneumonia of Anatolian goats)

KOLAYLI, C. and RAIF, M. in collaboration with ESIM, I, and ARAYICI E. (1935). *Rec. Méd.Vét* **CXI, 38,** and *Bull. Acad. Vét.* **VIII, 227.**

The first article deals with the symptoms and lesions, bacteriology, and infectivity of morbid materials and cultures.

Symptoms and lesions.—It is a disease of the winter and is characterised by pyrexia, cough, nasal discharge, inappetence, conjunctivitis, abortions, pleuro-pneumonia usually more marked in the right than in the left lung and death. In advanced cases areas of necrosis, which are not encapsulated appear in the lungs. Some goats escape death and become dangerous as carriers.

Bacteriology.—A pasteurella is recoverable in pure culture from affected lungs from the acute cases, and in admixture with contaminants from chronic cases, but not at all from cases of the fulminating type. This organism, which the authors name *B. bipolaris*

caprisepticus is the largest of bipolar organisms, pleomorphic, non-motile, non-sporing, and gram-negative. It is non-haemolytic and reduces litmus-milk but does not coagulate i Most strains produce indol but none hydrogen-sulphide. Growth in broth resembles that of most other pasteurella. On agar it develops in dew-drop-like colonies which coalesce to form a feebly shining film. The authors consider that its inability to grow on the Drigalski plate, its poorly saccharolytic properties and inagglutinability by five of the Salmonella sera further relegate it to the Pasteurella group as opposed to *S. para-B.* and related species.

Cultures are viable at 37°C. for 35 days and in Tuche's medium for 75 days. They are killed at 58°C. in one hour.

Infectivity.—Filtrates of morbid material, and pleural exudates are non-infective. Defibrinated blood gives rise to no more than a transient pyrexia. Saline emulsions of lungs obtained from acute cases of the disease and the supernatant fluid resulting therefrom after sedimentation are infective to goats by intra-pulmonary inoculation.

Cultures are fatal to mice, guinea-pigs, rabbits and pigeons, non-fatal to dogs and cats, and non-infective to fowls. The calf suffers from mere pyrexia after a subcutaneous inoculation of 1 c. c., but dies after an intravenous inoculation of 5 c. c. Cultures are fatal to goats by all the routes excepting the intra-tracheal one.

In the second article the prophylactic and curative measures are described. A formalised killed culture, a formalised saline emulsion of hepatised lung and pleural effusion, and dried powder made from the emulsion were tried. The emulsion was found to be the best, protecting all the fifteen vaccinated animals against contact infection.

Serum made from affected goats, given intravenously at 20 c. c. every three days, was found to have a curative effect. [V. R. R.]

Vitamins and Minerals in Poultry Nutrition. By E. M. CRUICKSHANK, *Nutrition Abstracts and Reviews*, Vol. 5, p. 1, 1935.

In this article the author has reviewed the question of vitamins and minerals in relation to poultry nutrition. The subject is discussed under two main heads. (1) The relation of minerals to poultry problems such as the calcium and phosphorus metabolism during the reproductive cycle, the requirements of the growing chick for these minerals, perosis, availability of various lime and phosphorus compounds, the effect of iodine supplement and the toxic action of fluorides, magnesium salts and excess of sodium chloride. A brief mention is also made of the effect of minerals on the resistance to disease. (2) The need of vitamins A, B, C, D and E for growing as well as adult birds. It is shown that deficiency of vitamin A appears to predispose the fowl to the onset of infectious diseases, that some of the vitamins of the B group are necessary for normal health and growth and that the requirement of vitamin D is very high during growth and still higher during egg production. In vitamin D deficiency in young chickens, gross skeletal changes such as beading of the ribs, deformity of the breast bone and enlargement of the epiphyses of the long bones are usually present. An enlargement of the parathyroid

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gland may occur and in rachitic birds the thyroid becomes extremely hyperplastic. It is realised that adequate attention has not yet been paid to the relation of vitamins and minerals to the incidence of diseases and the hope is expressed that further research in these lines may have a valuable practical application in reducing the high mortality which frequently occurs in poultry flocks.   [K. C. S.]

Protecting the natural flavour of Milk.   J. L. HENDERSON and C. L. ROADHOUSE
Hoard's Dairyman, Vol. 80, No. 19, October 10th, 1935.

For the consumption of fluid milk to be maintained and increased, the product must be kept uniformly palatable from day to day.

The authors summarise the results of recent investigations carried out by the United States Department of Agriculture on the sources of certain unpalatable flavours in milk.

The unpalatable flavours maybe brought about by feed by auto-oxidation of milk fat hastened by certain metals, by high temperature, by sunlight, by absorption of odours of some fruits, vegetables, chemicals or by rancidity caused by the enzyme lipase which splits fats into fatty acids having characteristic odours.

The natural flavour of milk may be protected by adjustment of the feed programme, so that animals do not have an access to feeds like, clover, corn silage, turnips, legume silage, etc., five hours before milking—such roughages should be fed immediately after milking. All types of dairy equipment should be obtained in non-corrodable metals [galvanised utensils so often seen in India are particularly bad—C. E. M.] and milk should be protected from exposure to direct sunlight through all stages of handling. [C. E. M.]

CORRESPONDENCE

DEAR SIRS,

Since the publication of my article on Pyosepticaemia of Calves in the September (1935) issue of *The Indian Journal of Veterinary Science and Animal Husbandry*, the organism which I described as a common cause of this disease has been serologically typed as *Salmonella enterditis*, var.*dublin*.

This is an interesting finding, as it appears the first record in India of the existence of this organism as the cause of disease in calves. A similar disease of calves has been encountered within recent years in a number of countries. One may quote from a recently published article by Lovell and Hughes, from the Research Institute of Animal Pathology, Royal Veterinary College :—

" Both *S. typhi-murium* (Bact. aertrycke) and *S. enteriditis* var. *dublin* have been incriminated in diseases of calves. The disease described by Thomassen [1897], was probably of this nature and as previously mentioned, the " para-colon " bacillus of Jensen which causes the well-known calf dysentery of Denmark is identical with the Dublin variety of *S. enteriditis*. *S. enteriditis*, or one of its varieties, has also been connected with calf disease in America [Meyer, Traum and Roadhouse, 1916], Kenya [Daubney, 1927], Germany [Proscholdt, 1931] and in Great Britain [Bosworth and Lovll, 1931]. Henning (private communication) has recently examined a number of strains of salmonella isolated from calves in South Africa and all but one were *S. enteriditis*, var. *dublin*. Judging from the literature, this is the common salmonella infection of calves and, incidentally, the same organism causes gastro-enteritis and continued fever in human beings ".

In the light of the foregoing observations, the finding of this organism in cattle in India, will most probably be of interest, not only to the veterinary, but also to the medical profession, on account of the implications involved in the presence of such an organism in mature milch cattle as well as in calves.

The part played by this organism in typhoid-like fevers of man, where attempted cultural examinations fail to reveal the presence of a true typhoid infection, may warrant serious consideration.

Yours faithfully,

J. F. SHIRLAW, M.R.C.V.S.,

Professor of Pathology, Punjab Veterinary College, Lahore.

18th March 1936.

BIHAR VETERINARY COLLEGE, PATNA.

The following notice has been received from the Principal, Bihar Veterinary College, Patna :—

A Post-Graduate class will be held at the Bihar Veterinary College from 1st July, 1936, for the advanced training of Veterinary Graduates in (1) Pathology and Bacteriology including Parasitology and *Postmortem* technique, (2) Histology, (3) Veterinary Hygiene, Dietetics and Genetics, (4) Meat and Milk Inspection and (5) Veterinary Medicine including parasitic, contagious and inanition diseases. The course will be for three months only for which a sum of Rs. 30 will be charged as tuition fee and Rs. 10 as admission fee. Seat rent for the hostel will be at the rate of Rs. 5 per month.

Private candidates desiring admission should apply to the Principal, Bihar Veterinary College, on or before the 15th June, 1936.

Further particulars, if required, may be obtained on application from the Principal.

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(Vol. VI, Part III.)

September, 1936.

The Editorial Committee of the Imperial Council of Agricultural Research, India, takes no responsibility for the opinions expressed in this Journal.

ORIGINAL ARTICLES

ORIGINAL ARTICLES

THE CURATIVE TREATMENT OF SURRA IN BOVINES

BY

S. K. SEN, M.Sc., F.R.E.S.,

Imperial Institute of Veterinary Research, Muktesar

(With Plate VII and three text figures)

INTRODUCTION

The present investigation originated out of an inquiry made by the Animal Health Division of the Council for Scientific and Industrial Research, Commonwealth of Australia, concerning the possibility of importing into that country certain types of Indian cattle under a guarantee to be given by the veterinary authorities in India that they are free from surra. A very real responsibility was involved in advising upon a matter of this kind, in view of the risk of veterinary officers inadvertently tarnishing the fair name of India by certifying animals as being entirely free from this disease, when such was not actually the case. At the same time, the inquiry brought into prominence the almost entire lack of authentic information concerning a dependable method of rendering affected cattle free from surra. Bovine surra, both in India and other countries, has never appeared to be deserving of the same degree of attention as most other diseases of cattle, this being attributable to the fact that, except in the few instances where the disease has been observed to progress to a fatal issue [Mahajan, 1934; Iyer and Sarwar, 1935], its symptoms have, as a rule, been confined to a negligible and erratic elevation of the body temperature. As remarked by Sheather [1934], "Cattle and buffaloes very seldom come as subjects for treatment owing to the fact that they may be infected without showing symptoms". Similarly, writing in 1921, Hornby went as far as to state: "I am aware of no literature which records recent outbreaks of surra among bovines, and this in spite of the wide distribution of the disease among horses and camels in countries where cattle and buffaloes are numerous. Consequently there has been no need for any work to be done on the treatment." The remarkable resistance displayed by bovines to the development of the clinical symptoms of surra is strikingly illustrated by the fact that "specially selected cattle from India, in the best of condition, have been found to be the carriers of trypanosomes in their blood after they have travelled to America" [Hoare, 1913]. Similar instances are recorded by Joseph [1928], according to whom the presence of surra trypanosomes in the circulating blood of

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bovines is not necessarily associated with an elevation of body temperature. There would thus appear to be some reason for doubt as to whether the parasite responsible for surra in bovines is referable to the species *Trypanosoma evansi* at all, and although in a recent communication, Rao and Mudaliar [1934] have produced some experimental evidence to show that this is so, it would seem that the final solution of the problem will not have been arrived at until further light has been thrown on the precise identity of the species of trypanosome involved in the occasional cases of fatal or acute surra in cattle. Should the parasite concerned in cases of this kind actually turn out, as a result of intensive morphological studies, to be indistinguishable from *T. evansi*, then it would be of considerable interest to ascertain the circumstances under which this parasite, which is usually innocuous for bovines, attains the status of a virulent variant for these animals.

Unfortunately, an investigation into problems relative to bovine surra is rendered difficult by the fact that there is no reliable method for the diagnosis of the disease itself. A perusal of the available literature shows that practically all workers are agreed as to the difficulty of diagnosing the disease in ruminants, and repeated reference has been made by them to the futility of declaring a bovine subject as free from surra from the mere fact that an examination of its blood smears has proved negative for the presence of surra parasites, for these may be so scanty that " microscopical examination of the blood carried out for long periods may fail to reveal the presence of trypanosomes " and even when the temperature is high " microscopical examination of the blood may fail " [Sheather, 1934]. The difficulty of diagnosing bovine surra with certainty has also been referred to by Bakker [1925] in the Dutch East Indies, while Gaiger [1911], in India, even declared that " ordinary microscopic examination of the blood of cattle for *T. evansi* in India is useless, the parasites are so few in number that they have never been so seen ". Several workers have, therefore, mentioned the desirability of diagnosing surra in bovines by means of the so-called biological tests, that is, by the sub-inoculation of the suspected blood into small animals [Doeve, 1918; Hornby, 1921; Sheather, 1934], although Gaiger [1911] would even regard this method of diagnosis as " obviously impracticable " but feels " almost certain that given time, money, and opportunities for experiment, other means might be devised ". In referring to the impracticability of the biological test, Gaiger had presumably in mind the difficulty of conducting large-scale sub-inoculations under the conditions obtaining in the field when one has to deal with an actual epizootic of cattle surra.

A reference may be made at this point to four other well-known methods that have been recommended from time to time for the diagnosis of surra, namely, Formol-gel [Knowles, 1924, 1925] and the Mercuric Chloride [Barnett & Kenny, 1928] tests and the method of diagnosis based upon the so-called Adhesion Phenomenon [Davis and Brown, 1927; Duke and Wallace, 1930]. Apart from the fact

that there is little evidence on record to show that any one of these can be utilized with success in the diagnosis of cattle surra, it still remains to be unequivocally established that the reactions obtained by these methods are not essentially group, as opposed to specific, reactions. The diagnostic value of Adhesion Phenomenon was repeatedly tested at Muktesar upon rats artificially infected with *T. evansi*, but the results were found difficult to interpret. As to the Complement Fixation test, however, Randall [1934] records having employed this successfully in detecting the infection in suspected horses in the Philippines, where *T. evansi* is the only known pathogenic type of trypanosome—a fact which evidently largely contributed to the deduction that the reaction obtained in these cases was not due to a species of trypanosome other than *T. evansi*.

In view of the uncertainty of the results to be expected from these tests, it was not considered worth while to employ them in the present trials, but to adhere to the established methods of diagnosis, as will be described later in this paper.

THE CHEMOTHERAPY OF BOVINE SURRA : A RÉSUMÉ OF THE LITERATURE

While, as would appear from what has been stated in the preceding section, there is a concensus of opinion concerning the unreliability of the methods hitherto used in the diagnosis of bovine surra, nevertheless, some workers would appear to hold that a permanent recovery in this condition is possible, although their views in this respect do not appear to be based on any extensive experimental data. Thus, Doeve [1917] refers to complete recovery having occurred in bovines when they were well fed and kept indoors, so that re-infection could not take place. Again, Leger and Vienne [1919] quote Lingard as having stated that recovery is the rule among cattle in India, infected with surra. Similarly, Broudin [1927] mentions the occurrence of spontaneous recovery in bovines if conditions are favourable.

Mesnil and Leger [1912] would appear to furnish somewhat more definite, although indirect, information upon the possibility of rendering cattle permanently free from surra. These authors quote Wryburg [1907] as having stated that two zebus which had recovered from surra in Sumatra were immune for a period which did not exceed two years, and Mesnil had previously [1910] recorded the case of another bovine that retained its immunity against a Mauritius strain for a period of more than two years. These observations would seem to imply that in the case of a " cured " bovine, a relapse is not likely to occur within two years from the date of its recovery, and one may assume that an animal that has not shown signs of relapse during this period is not likely to do so during the rest of its life.

Douwes [1923], in Java, claims to have effected complete cure with Naganol in seven out of eleven buffaloes suffering from surra, the blood of all cured animals remaining negative for surra trypanosomes " as long as they were under observation ". Each animal received a single intravenous injection of 3 or 5 grms. of

of the drug in a 5 per cent solution and all the animals were kept in dark, fly-free stables. An unsatisfactory feature of these experiments, however, was that, except one animal, none showed trypanosomes in blood before treatment, diagnosis having been made from symptoms alone, and the author states that "the buffalo is a difficult animal to deal with". Three Bengal oxen showing surra trypanosomes in their blood were also treated by him with the same drug and their blood remained negative for one month after a single injection of 5 grms. It is of interest that in the Annual Report of the Department of Agriculture, Java, 1922, Naganol is mentioned as having yielded "encouraging results in acute surra in bovines".

Lagas [1926], in Sumatra, treated 811 buffaloes "prophylactically" with Naganol, from October 1924 to April 1925. Sixty-four of these were healthy carriers, ninety-four showed clinical symptoms of the disease and forty-two showed the parasites in their circulating blood. During this period, forty-nine of the treated buffaloes died from various causes and nine showed trypanosomes in their blood, the intervals between appearances of the parasites ranging from 5 weeks to 10 months. Eight of the animals received a second dose of 2-4 grms. of Naganol and none of these relapsed "up to the present" (the article was published in April 1926).

In a recent paper, Bakker [1932], in the Dutch East Indies, has recorded having successfully used Naganol in 5-grm. doses in the treatment of 130 adult buffaloes affected with surra. He also reports having obtained encouraging results in the treatment of surra in cattle with the same drug.

A perusal of the available records of work carried out in India upon the treatment of bovine surra shows that some of the earliest attempts to cure the disease in this country were made by means of atoxyl and certain indigenous remedies [Chetti, 1922 ; Rao, 1923], but these came to be gradually replaced by tartar emetic, which held the ground for some length of time and is in favour with certain workers even to this day. Concerning this drug, however, Edwards [1926], as a result of experiments carried out at Muktesar, observes that although a single injection of the drug at the rate of 5 c.c. of a 3·2 per cent solution per 100 lb. body weight frequently produces a recovery, it is known "that the parasites remain thereafter in a state of latent activity". This conclusion has quite recently been confirmed by Sarwar [1936], who found that cattle treated for surra with tartar emetic continued to harbour the parasites, as determined by biological tests. On the other hand, Iyer and Sarwar [1935] obtained what would appear to be consistently satisfactory results in combating a natural outbreak of bovine surra in the Punjab, from the use of Naganol administered intravenously at the rate of 10 to 20 c.c. of a 10 per cent solution, depending upon the size of the animal concerned. Similar results had also been previously reported by Mahajan [1934]
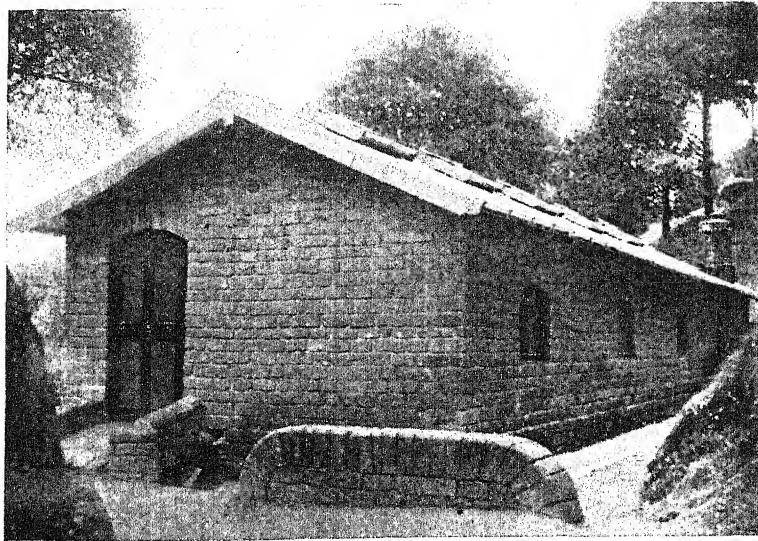
Fig. 1.—A view of one of the fly-proof stables, with the double-door closed.
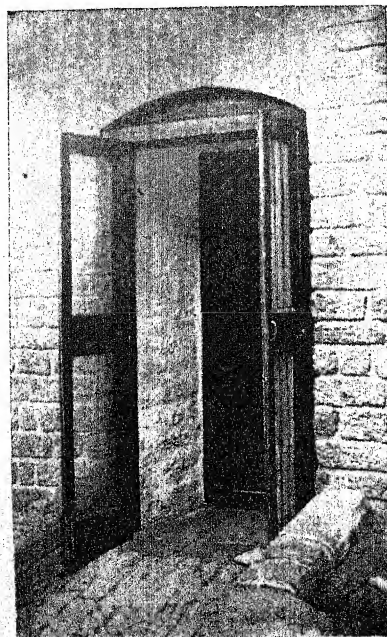
Fig. 2.—A view of the double-door while open.

from Hyderabad-Deccan. As will be seen from what is stated in the following section, Naganol was also the drug used throughout the experiments described in the present paper.

Rodenwaldt and Douwes [1922], however, would appear to discredit all reports of alleged " cures " in buffaloes and in other animals suffering from chronic surra infections, for according to these authors, such animals frequently show long remissions during which " the trypanosomes are so scanty in the blood that they may be undiscoverable ". They refer to the futility of studying the action of Naganol upon surra in isolated cases and stress the importance of carrying out large-scale experiments before any conclusions can be drawn regarding the efficacy of this drug for surra in buffaloes.

It would seem that the final conclusion to be arrived at in regard to the prospects of obtaining cures in surra in the bovine subject by means of drug treatment will have to be based upon the results of a series of large-scale experiments, as suggested by Rodenwaldt and Douwes [1922], with due regard to the possibility that a proportion of the reported relapses in the past may have been, in reality, cases of re-infection contracted through the agency of insect vectors. As will be seen presently, this possibility was borne in mind when planning the present investigation.

MATERIAL AND METHODS

In the present investigation a total of fifteen animals, comprising six hill bulls and nine buffaloes, were employed. As will be explained later, two out of the nine buffaloes had been actually brought under experimentation long before the trials originating out of the Australian inquiry were commenced, while two buffaloes died and one buffalo had to be discarded and replaced by another during the progress of the investigation. Except for these six cases, the observation period in the case of all others, was about one year and a half.

The experimental animals were housed in two especially-constructed fly-proof stables (Plate VII, fig. 1) in order to prevent them from contracting a natural infection through the agency of biting flies. At the entrance, the stables were provided with double-doors (Plate VII, fig. 2) designed to prevent any possibility of access of flies while the attendants are entering or leaving the premises. The windows and the ventilators were covered with wire-netting, while all outlets for the drainage of urine and stable washings were suitably screened or plugged up. It is almost needless to emphasize the importance of protective measures of this kind, for, as Musgrave and Clegg [1903] have remarked, " to obtain satisfactory results such a structure is an absolute necessity ".

The strain of *T. evansi* used in these trials had been repeatedly proved to be highly virulent on the equine subject and had been maintained for a number of years in the laboratory by passing through rabbits and guinea-pigs.

Prior to the infective inoculation, each animal was kept under observation for about two weeks, and during this period its blood was sub-inoculated once into rabbits and was also daily examined in wet smears for the presence of surra trypanosomes, and it was brought under experimentation only after it had definitely proved to be free from surra.

The drug used in these trials was Naganol and the dosage employed was at the rate of 5 grms. per 1,000 lb. bodyweight in a 10 per cent aqueous solution administered intravenously, this dosage having been based upon the results previously obtained in the laboratory in the treatment of equines, artificially infected with surra. In view of the lower susceptibility of cattle to surra, Baermann [1923] would appear to regard it as probable that a small dose of Naganol is sufficient for bringing about a cure in bovine surra, and Iyer and Sarwar [1935] have actually used what would appear to be smaller doses of the drug, with apparent success, in the treatment of this disease. In the present trials, however, it seemed desirable to use the maximum therapeutic dose of the drug, in conformity with Ehrlich's well-known dictum *therapia sterilisans magna*, so as to prevent any possibility of the development of Naganol-resistant strains of *T. evansi*. As will be seen from Tables I and II, seven animals received a single injection of Naganol, whilst two received three injections at intervals of three months, the remaining six animals—two bulls and four buffaloes—were kept as " controls ".

At the outset, it was realized that the criterion to be laid down for assessing the value of a therapeutic remedy for bovine surra was the actual extent of the sterilization of the circulating blood brought about by the treatment, quite irrespective of any apparent amelioration of the febrile symptoms which, as has already been indicated earlier in this paper, has not always been found to bear any definite relationship to the actual progress towards recovery.

For the detection of the parasites in the circulating blood, three methods were employed : (1) Daily examination of blood smears for a period of about six months from the date of commencement of the experiments, and later, weekly and also on the appearance of any suspicious clinical symptoms, it having been considered improbable that the parasites, if present, would escape detection on blood smear examination when this was continued over as long a period as one year and a half ; (2) periodical—generally at three-monthly intervals—sub-inoculation of about 2 to 5 c.c. of blood into rabbits ; and (3) sub-inoculation of about 200 c.c. of blood from each animal into susceptible equine subjects at the termination of the experiments. The observation period in the case of the sub-inoculated rabbits ranged from four weeks to one month and that in the case of the sub-inoculated ponies was about three weeks. In addition, a steady and consistent gain in body weight shown by a treated animal, as contrasted with the loss in weight sometimes observed in the case of untreated animals, was regarded as additional evidence of the efficacy of the remedy.
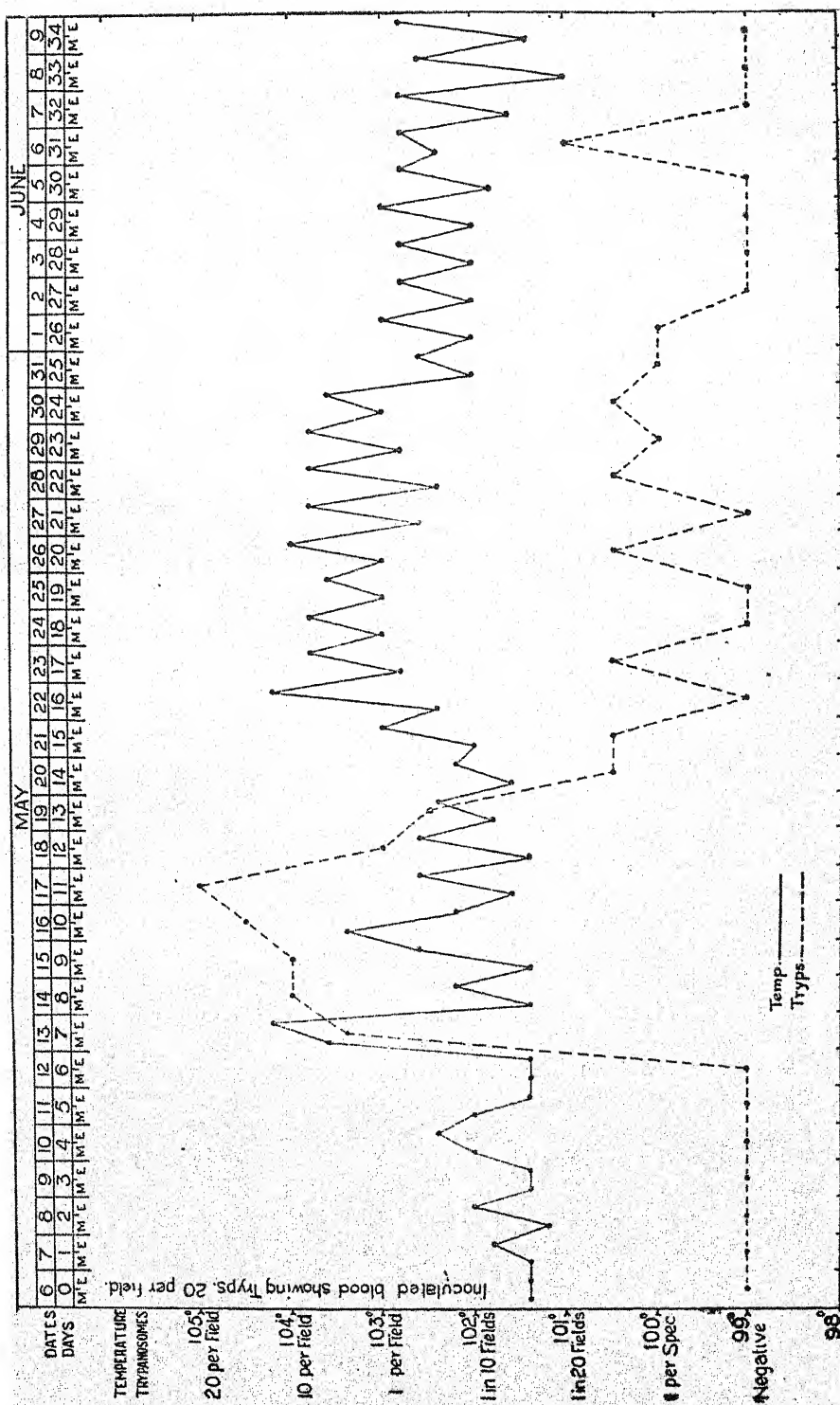
Buffalo No. 6 (Control).

FIG. 1.

The Course that Surra runs in Bovines and Observations upon the Effect of Naganol in the Control of this Condition

A perusal of the available literature shows that, while a considerable amount of valuable observations has been made upon the clinical symptoms of equine surra, the information on record concerning the course run by surra in bovines is very fragmentary. The present trials provided an excellent opportunity of maintaining an accurate record of the symptoms exhibited by the " control " animals during, as already mentioned, an observation period of a year and a half and a brief account of these symptoms may be appropriately included here.

Fig. I, which represents the chart of " Control " Buffalo No. 6, is illustrative of the course of the disease as usually observed in bovines. As will be seen, this animal developed the infection after an incubation period of about one week and during the following five days it showed consistently large numbers of parasites in the circulating blood, with a corresponding elevation in the body temperature. During the eighteen days that followed, the parasites continued to make their appearance in an erratic manner, until after a sudden increase in their numbers about the 30th day from the date of infective inoculation, they disappeared altogether. Curiously, although the parasites were never again seen in blood smears from this animal, nor was there a suspicious rise in its body temperature, its blood proved infective on sub-inoculation into rabbits, at the end of the second month from the date on which the parasites were last encountered on smear examination (Table I).

TABLE I

Summary of observations upon "control" animals

Serial No.	Animal No.	Dates of infection	Dates on which the results of blood smear examination were positive	Dates of sub-inoculation into rabbits, Positive results are indicated by asterisks (*)	Remarks
1	Buffalo 6	May 6, 1932	May 13-21, 23, 26, 28-29, June 1, 6, 1932.	April 22, August 6*, November 6, 1932; February 6, 1933.	Died February 27, 1933 of haemorrhagic septicaemia.
2	Buffalo 4	September 19, 1932.	September 25-29, October 8-9, 20-21, November 18, 1932.	December 21, 1932; March 21*, June 20, 1933.	Discontinued December 20, 1933.
3	Buffalo 1849	April 29, 1930	May 6-8, July 19, 25, August 11, 1930.	May 17, September 8, 1931; January 21, August 6, November 6, 1932; February 6, 1933.	Discontinued December 20, 1933.
4	Buffalo 1 B.	May 6, 1932	May 11-14, 28-29, 1932	. .	Discarded June 15, 1932.
5	Bull 1035	May 6, 1932	May 10-14, August 19, 1932.	April 22, August 6*, November 6, 1932; February 6, 1933.	Discontinued December 20, 1933.
6	Bull 1055	May 6, 1932	May 11-13, 1932	April 22, August 6, November 6, 1932; February 6, 1933.	Discontinued December 20, 1933.

The protocol of Buffalo No. 4 (Table I) illustrates the difficulty of declaring an animal as cured on the basis of results obtained from a single series of sub-inoculations. As will be seen, this animal developed the infection after an incubation period of about one week and it showed the parasites in its blood on three occasions during the period of about one month and a half that followed. Two rabbits were sub-inoculated from this animal about the third month from the date of infective inoculation, but neither developed the infection. On the other hand, when the sub-inoculation was repeated about three months later, the results were positive. The spasmodic behaviour of the parasites in bovines is likewise characteristically illustrated in the case of Hill Bull No. 1035 (Table I). In this case, the parasites, after a continued absence in the circulating blood for a period of more than three months, suddenly re-appeared for a single day and were never encountered again on blood smear examination.

The history of Buffalo No. 1849 provides a feature of special interest in that it was kept under observation for as long a period as three years and eight months (29th April 1930 to 20th December 1933). This animal, which represented the "control" to Buffalo No. 1897 (*infra*), had been actually brought under experimentation long before the main series of experiments originating out of the Australian inquiry were initiated. On referring to Table I, it will be observed that in the case of this animal, the parasites were encountered on four occasions on blood smear examination, at intervals of 71, 5 and 16 days respectively. Sub-inoculation from this animal into rabbits was not commenced until after about one year from the date of infective inoculation and was repeated on five occasions at intervals of 113, 134, 196 and, later, at three-monthly intervals, but the results were all negative. It is noteworthy that neither in the case of this animal, nor in the case of Buffalo No. 1897, which represented its pair, was any special precautionary measure adopted to protect them from bites of flies, until after an expiry of about two years from the date of infective inoculation, when they were housed, along with the other animals, in fly-proof stables.

Bull No. 1055 represents an extreme instance where the inoculation of surra parasite failed to produce any results whatsoever, beyond the usual reaction following upon the incubation period of the disease.

Buffalo No. 1 B had been originally included in the series of "controls", but having proved unsuitable for use, it had to be discarded and replaced, about three months later, by Buffalo No. 4 to which a reference has already been made. As will be seen from Table I, this animal relapsed after an apparent recovery lasting for only about 13 days.

It is noteworthy that while the initial reaction of the disease was definitely a thermal reaction associated with the occurrence of varying numbers of parasites in the circulating blood, the relapses, on the other hand, were, as a rule, devoid

of any febrile symptoms and were characterized by the appearance of the parasites only. This would appear to be in striking contrast with what one observes in the case of equine surra, in which the succession of parasitic relapses is almost invariably associated with elevation of body temperature.

In regard to the animals treated with Naganol, a single injection of the drug, administered at the rate of 5 grms. per 1,000 lb. body weight, invariably resulted in a complete sterilization of the circulating blood, as judged by the results of blood-smear examination and of periodical sub-inoculation of blood into small animals (Table II). In all cases, the parasites disappeared from the circulating blood within 24 hours of the injection of the drug (Figs. 2 and 3) and did not appear again during the observation period of (in the majority of cases) one year and a half. It is to be mentioned, however, that Buffalo No. 1574 died of enteritis and debility about 11 months prior to the date (December 20, 1933) on which it was due to be discontinued and that no opportunity, therefore, occurred for testing the infectivity of its blood on a susceptible equine subject. Nevertheless, the post-treatment period of about eight months and a half during which it was kept under observation would appear for all practical purposes to be sufficiently long for assessing the value of the remedy in this case.
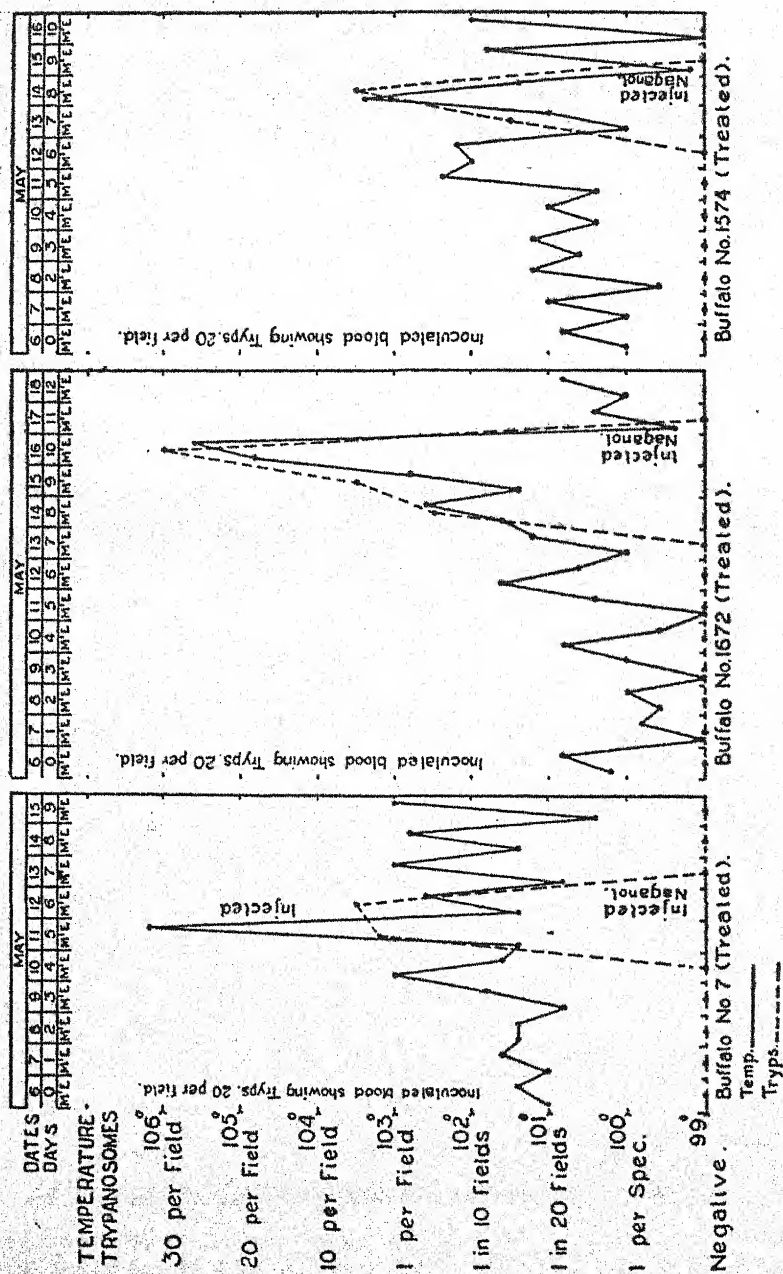
FIG. 2.

TABLE II

Summary of observations upon treated animals

Serial No.	Animal No.	Date of infection	Date of treatment with Naganol	Dates on which the results of blood smear examination were positive	Dates of sub-inoculation into rabbits (results all negative)	Remarks
1	Buffalo 7	May 6, 1932	May 12, 1932.	May 11, 12, 1932.	April 22, August 12, November 12, 1932; February 15, 1933.	Discontinued December 20, 1933.
2	Buffalo 1673	May 6, 1932	May 16, 1932.	May 14, 15, 16, 1932.	April 22, August 16, November 16, 1932; February 17, 1933.	Discontinued December 20, 1933.
3	Buffalo 1574	May 6, 1932	May 14, 1932.	May 13, 14, 1932.	April 22, August 14, November 14, 1932.	Died January 30, 1933 of enteritis and debility.
4	Buffalo 12	May 6, 1932	May 14, August 14, November 14, 1932.	May 12, 13, 14, 1932.	April 22, August 14, November 14, 1932; February 17, 1933.	Discontinued December 20, 1933.
5	Buffalo 1897	April 29, 1930	May 6, 1930.	May 6, 1930	August 31, September 25, 1930; May 17, September 10, 1931; January 21, August 16, November 16, 1932; February 17, 1933.	Discontinued December 20, 1933.

Serial No.	Animal No.	Date of infection.	Date of treatment with Naganol	Dates on which the results of blood smear examination were positive.	Dates of sub-inoculation into rabbits (results all negative)	Remarks.
6	Bull 1064	May 6, 1932	May 12, 1932.	May 10, 11, 12, 1932.	April 22, August 12, November 12, 1932; February 15, 1933.	Discontinued December 20, 1933.
7	Bull 1047	May 6, 1932	May 12, 1932.	May 11, 12, 1932.	April 22, August 12, November 12, 1932; February 15, 1933.	Discontinued December 20, 1933.
8	Bull 1059	May 6, 1932	May 12, 1932.	May 11, 12, 1932.	April 22, August 12, November 12, 1932; February 15, 1933.	Discontinued December 20, 1933.
9	Bull 1038	May 6, 1932	{ May 14, August 14, November 14, 1932. }	May 11, 12, 13, 14, 1932.	April 22, August 14, November 14, 1932; February 17, 1933.	Discontinued December 20, 1933.
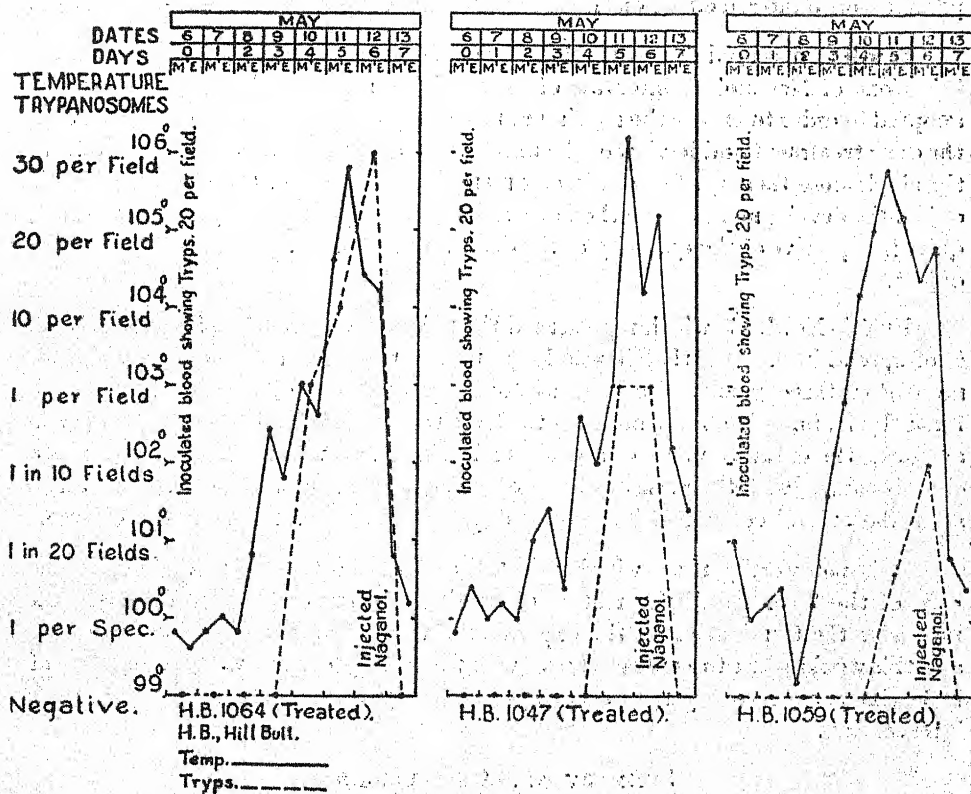
Fig. 3.

A reference to column 4 in Table II and to Figs. 2 and 3 will show that, while in every one of the artificially infected animals, the disease was allowed to be fully established, in no instance did the infection proceed beyond 48 hours when treatment was applied. The object of this was to make sure that recovery was actually brought about as a result of drug intervention and not, as has frequently been observed to be the case, by a process of spontaneous disappearance of the parasites. However, what appeared to be the conclusive evidence of the efficacy of Naganol was provided by the fact that in none of the treated animals were the parasites encountered either on smear examination or as a result of sub-inoculation.

It will be observed (Table II) that two of the animals received a course of three injections of Naganol at intervals of about three months. This procedure was adopted in order to ensure the maximum possibility of success being obtained with the drug treatment and in view of the uncertainty of the results to be expected from the single-dose therapy, for, as already mentioned, the present trials had for their object the development of a method of rendering cattle permanently free from surra, quite irrespective of the quantity of the drug required for the attainment of this object.

Buffalo No. 1897, which represented the pair to " Control " Buffalo No. 1849, is of special interest in that it was kept under post-treatment observation for more than three years and a half, without any evidence of relapse being exhibited by it, for it is almost needless to stress the importance of the time factor in assessing the curative value of a drug for bovine surra which is so liable to run a chronic course, with the parasites unaccountably appearing in the circulating blood after the animal concerned has, to all appearances, recovered from the disease.

It is noteworthy that, whether in the case of the treated animals or in the case of the " controls," the results of sub-inoculation of blood into susceptible ponies at the termination of the experiments were of an entirely negative order, and this would seem to confirm the view expressed by certain workers (ante) that in bovine surra recovery may occur spontaneously without the necessity of drug intervention.

SUMMARY AND CONCLUSIONS

This investigation was undertaken to test the possibility of curing bovine surra with certainty by means of Naganol. For this purpose, a total of fifteen animals, comprising six bulls and nine buffaloes, were artificially infected with a laboratory strain of *Trypanosoma evansi* and housed in fly-proof stables. Out of the fifteen animals, four bulls and five buffaloes were treated intravenously with one to three doses of Naganol at the rate of 5 grms. per 1,000 lb. body weight and the remaining six animals were kept as " controls ". One treated and one untreated buffalo were kept under observation for about three years and a half, while the observation period in the case of the remaining animals ranged from thirty-nine days (in one

instance) to about one year and a half.   During these periods, one " control " bull and all the four " control " buffaloes showed the parasites at various intervals in their circulating blood, as revealed by the results of blood smear examination or of the sub-inoculation of their blood into rabbits, the maximum period up to which evidence of infection was thus obtained having been about six months (Buffalo No. 4) from the date of infective inoculation. The treated animals, however, remained entirely free from infection after the initial sterilization of their blood by drug injection. At the termination of the experiments, about 200 c.c. of blood from each of the "controls," as well as from each of the treated animals, was sub-inoculated into susceptible equine subjects, but the results were of an entirely negative order, and from this one would conclude that in bovine surra spontaneous recovery is the rule rather than the exception.   Nevertheless, the fact that some of the " controls " continued to show the parasites periodically for some length of time after receiving the infective inoculation would appear to point to the advisability of treating all actual or suspected cases of bovine surra with Naganol before they are declared to be definitely free from the disease.

While a single dose of Naganol proved effective in at least seven out of the nine cases treated with this drug, it would seem very desirable, in view of the observations recorded by Lagas [1926], to repeat the dosage once, and, if possible, even twice, at intervals of one month, particularly when the animal concerned is intended for export to countries known to be free from surra.

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COMPOSITION OF COLOSTRUM FROM THE MONTGOMERY COWS

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(With one text-figure)

The milk produced by the cow during the first few days after calving is some-times referred to as ' beastings ' or ' green milk ' but more commonly as colostrum. The composition, condition, colour, taste and smell of the milk immediately after parturition differs considerably from that of normal milk which the cow gives, say, a week later. The change to normal takes place gradually and is complete within 5 to 6 days, although some of the ingredients such as fat and milk sugar change over very rapidly to their normal proportions. The pungent taste and slimy appearance of the colostrum do not last for more than a couple of days. The dairy herd attached to the Agricultural College, Lyallpur, consists of the cows of the Montgomery breed alone and true colostrum as generally obtained from these animals is usually yellow and very often deep yellow in colour.

Our knowledge about the true function of colostrum in the nutritional meta-bolism of the new born animals is limited. It is generally supposed to act as a laxative and facilitates the early passage of the meconium from the intestinal tract of the infant animal. Another rôle sometimes attributed to this product is its high concentration of nutritive elements in a form easily digested by the young animals [Ling, 1930]. Earle and Gamble [1934] working on young foals have discovered yet another function for this material. The results of their investi-gation have shown that, in at least some species, colostrum serves to immunize the new born animal passively against bacteria for which its dam has already acquired immunity.

Until recently, the belief was commonly held that the milk is secreted pri-marily for the nourishment of the calf and scientists and laymen alike believed that full development of the mammary glands and the actual secretion of milk could be brought about only by the normal processes occurring during pregnancy. The fact that the nature of the milk immediately after calving was different from that of the normal milk has been attributed to the immediate specific requirements of the newly born animal. Recent work carried out by the Bureau of Dairy In-dustry, U. S. A., as reported by Evans [1934], has, however, shown that pregnancy

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is not necessary for full development of the mammary glands and milk secretion and that by the injection of extracts of the anterior pituitary gland into a virgin dairy heifer, a daily milk yield of 15 to 18 pounds was obtained and maintained over several months after the injections had ceased. No mention is, however, made of the composition of the milk obtained. It would be interesting to know if the milk was uniformly alike in this respect throughout the entire period of lactation or if the composition of the first milk obtained from the virgin heifer differed in any way from the milk obtained, say 3 or 4 days after, *i.e.*, if any colostrum was secreted. The absence of any difference between the two would lend a strong support to the belief that colostrum with its characteristic properties which are so widely different from those of the normal milk is elaborated simply to meet the requirements of the newly born animal.

The object of the present note, however, is not to say anything on the biological functions of colostrum, but to present a certain amount of analytical data with regard to colostrum secreted by Montgomery cows.

The figures presented in the following tables give the analytical composition of the colostrum and the milk from three different cows of the Montgomery breed kept at the Agricultural College Dairy Farm, Lyallpur. The percentage composition of the colostrum from all the three animals, immediately after calving, was as follows :—

	Specific gravity at 60°F	Total solids	Fat	Milk sugar	Acidity	Total proteins	Casein	Albumin and Globulin	Ash
Cow 1 .	1·056	21·90	4·3	2·80	0·315	13·45	3·42	10·93	0·80
Cow 2 .	1·058	25·78	6·9	3·44	0·446	14·38	6·74	7·59	1·34
Cow 3 .	1·058	19·98	2·7	3·67	0·342	11·84	3·57	8·49	1·55
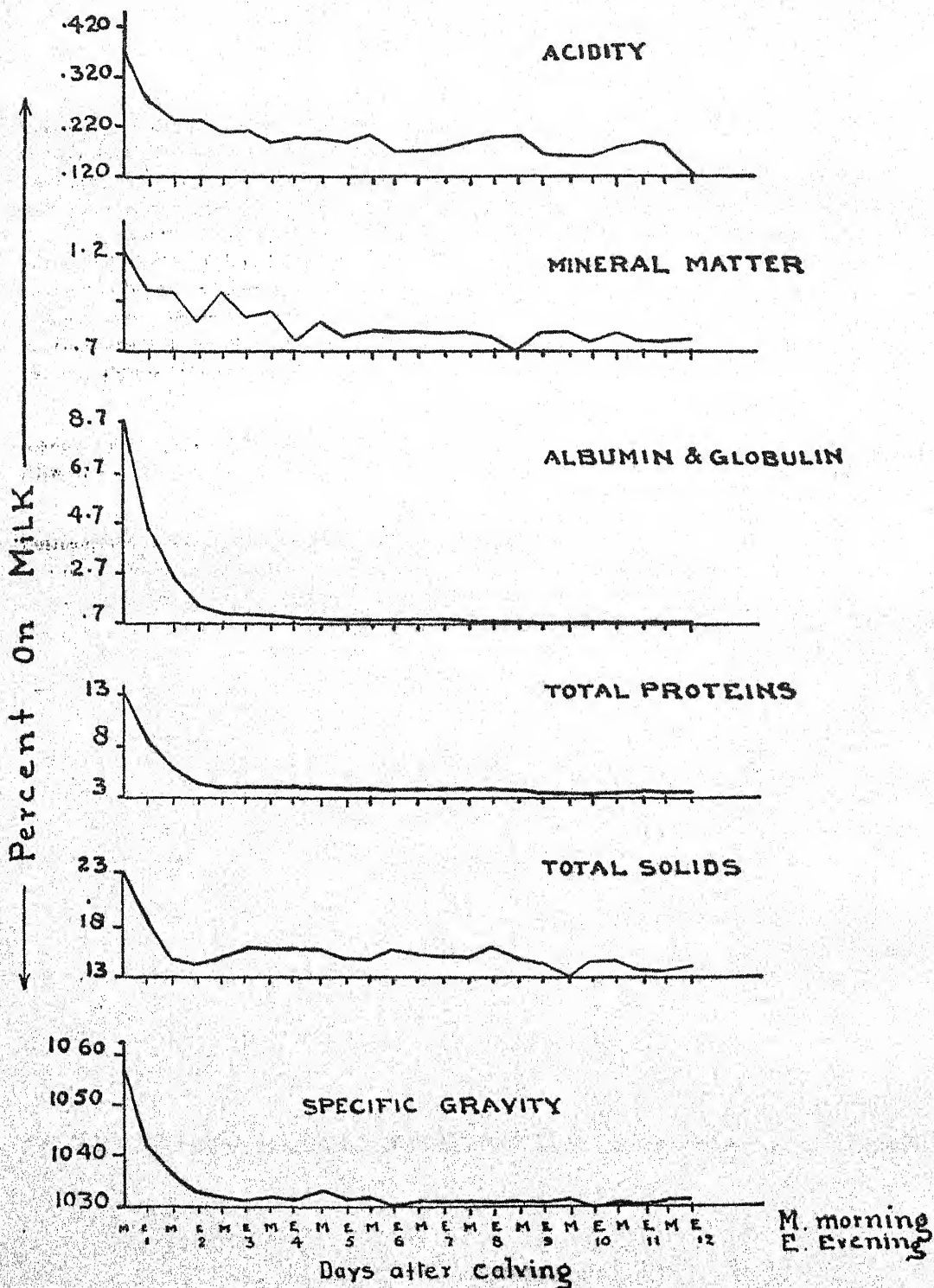
Diagram Showing average composition of milk on
12 consecutive days after calving.

It will be noted that the colostrum from Cow No. 2 contains a much greater amount of total solids than that from both of the other animals and the difference is due to the greater amount of fat and casein present in the colostrum of this animal. The other ingredients do not show so wide a difference. The complete analytical data, showing the gradual change from colostrum to normal milk in the case of all the three animals, is presented in the three Tables I, II, and III that follow. Table IV gives the average figures from the three individual animals and these are represented graphically in the diagram. The changes suffered by the individual ingredients may be summarised thus :—

(1) The initial high figures for total solid, acidity, and mineral matter decline rapidly and reach to an almost constant value within 24 hours.

(2) Total proteins, decrease gradually to about 25 per cent of their original value in 36 hours. The greatest change, however, takes place in the amount of albumin and globulin, which decrease to about 6 per cent of the initial figure. Casein, except in the case of animal No. 2 where the initial figure was very high, does not diminish to any appreciable extent. This diminution in the amount of water-soluble proteins seems to synchronise with the appearance of digestive enzymes in the stomach of the calf.

Table V gives the refractive index, saponification value and the Iodine value of the fat obtained from daily milk over a period of 7 days after calving. The figures for the refractive index and Iodine value for fat from colostrum are almost identical with those of fat from normal milk ; saponification value, on the other hand, shows a gradual increase, indicating that the fatty acids of high molecular weight contained in the fat are slowly changing to those of low molecular weight.

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TABLE I

Colostrum analysis (First Period)

Serial No.	Date and time of sampling		Specific Gravity at 60°F Per cent	Total Solids Per cent	Fat Per cent	Acidity Per cent	Total Proteins Per cent	Albumin and Globulin Per cent	Casein Per cent	Lactose Per cent	Mineral matter Per cent	Remarks
1	20th Novr. 1928	E	1·056	21·90	4·3	0·315	13·42	10·03	3·42	2·80	·803	E. Evening milk.
2	21st Novr. 1928	M	1·041	17·20	5·8	0·230	8·82	5·40	3·40	3·82	·788	M. Morning milk.
3	Do.	E	1·034	13·99	4·4	0·221	5·61	2·21	2·68	3·29	·888	
4	22nd Novr. 1928	M	1·032	12·99	3·2	0·207	4·33	1·34	2·59	3·97	·762	
5	Do.	E	1·030	12·32	4·3	0·212	4·00	1·46	2·53	3·86	·886	
6	23rd Novr. 1928	M	1·032	13·80	4·3	0·216	4·13	1·14	3·17	3·67	·898	
7	Do.	E	1·030	14·80	5·5	0·185	4·15	0·98	3·28	4·01	·828	
8	24th Novr. 1928	M	1·032	15·00	5·2	0·185	4·31	1·14	3·28	4·12	·818	
9	Do.	E	1·032	14·20	5·2	0·207	4·27	0·94	3·28	4·09	·878	
10	25th Novr. 1928	M	1·033	13·20	4·7	0·221	4·29	0·92	3·22	4·09	·772	
11	Do.	E	1·033	14·30	4·0	0·198	4·11	0·87	3·24	4·09	·880	
12	26th Novr. 1928	M	1·031	15·50	5·4	0·180	3·91	0·87	3·06	4·12	·910	
13	Do.	E	1·030	15·30	5·8	0·189	3·71	1·05	3·04	3·75	·774	
14	27th Novr. 1928	M	1·030	15·10	5·4	0·185	3·78	0·92	2·88	4·26	·872	
15	Do.	E	1·030	14·90	6·5	0·230	3·80	1·04	2·93	3·70	·762	
16	28th Novr. 1928	M	1·031	15·80	5·6	0·234	3·80	1·04	2·93	3·89	·844	
17	Do.	E	1·030	14·70	5·6	0·194	3·44	0·87	2·81	4·19	Spoiled	
18	29th Novr. 1928	M	1·030	14·30	4·7	0·198	3·51	0·78	2·77	3·94	·814	
19	Do.	E	1·030	13·36	4·5	0·198	3·55	0·78	2·86	4·66	·924	
20	30th Novr. 1928	M	1·030	14·00	4·9	0·189	3·84	0·83	2·88	4·43	·820	
21	Do.	E	1·030	15·20	6·1	0·170	3·62	0·83	3·06	4·39	·824	
22	1st Dec. 1928	M	1·032	14·10	4·7	0·171	3·80	0·83	3·06	4·66	·788	
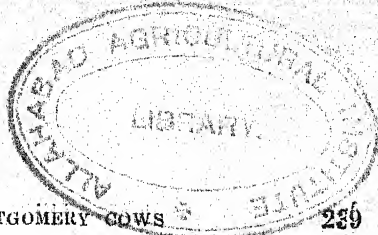
TABLE II

Colostrum analysis (Second Period)

Serial No.	Date and time of sampling		Specific Gravity at 60°F	Total Solids Per cent	Fat Per cent	Acidity Per cent	Total Proteins Per cent	Albumin and Globulin Per cent	Casein Per cent	Lactose Per cent	Mineral matter Per cent	Remarks
1	22nd Jan. 1929	M	1·058	25·78	6·9	0·446	14·38	7·59	6·74	3·44	1·336	M. Morning milk.
2	Do.	E	1·040	19·57	6·7	0·279	7·68	3·91	4·09	4·33	0·986	E. Evening milk.
3	23rd Jan. 1929	M	1·038	14·67	3·3	0·252	5·38	1·72	3·84	4·90	·964	
4	Do.	E	1·032	13·85	4·2	0·252	4·15	1·23	2·95	4·22	Spoiled	
5	24th Jan. 1929	M	1·030	16·36	6·6	0·216	4·44	1·03	3·42	4·63	Do.	
6	Do.	E	1·027	18·62	8·2	0·194	4·06	0·92	3·17	5·36	·857	
7	25th Jan. 1929	M	1·030	17·15	6·4	0·203	4·56	0·94	3·62	5·12	·887	
8	Do.	E	1·030	16·77	6·1	0·173	4·06	0·94	3·28	5·73	·693	
9	26th Jan. 1929	M	1·031	16·84	6·6	0·182	4·18	0·89	3·24	6·04	·887	
10	Do.	E	1·030	16·50	5·7	0·176	3·95	0·88	3·46	6·14	·744	
11	27th Jan. 1929	M	1·031	15·47	5·0	0·171	4·11	0·83	3·13	5·63	·774	
12	Do.	E	1·029	16·09	6·8	0·153	3·75	0·74	2·97	5·12	·742	
13	28th Jan. 1929	M	1·032	15·12	5·1	0·153	3·86	0·83	2·95	5·20	·838	
14	Do.	E	1·031	15·20	6·1	0·207	3·60	0·72	2·99	4·63	·804	
15	29th Jan. 1929	M	1·029	14·40	5·4	0·171	3·62	0·72	2·90	4·76	·944	
16	Do.	E	1·032	16·12	7·1	0·216	3·55	0·72	2·83	4·97	·642	
17	30th Jan. 1929	M	1·031	14·36	5·0	0·221	3·66	0·67	3·06	5·24	·600	
18	Do.	E	1·033	15·41	5·9	0·167	3·44	0·69	2·72	5·12	·810	
19	31st Jan. 1929	M	1·029	13·40	3·6	0·162	3·64	0·67	2·66	5·20	·754	
20	Do.	E	1·031	15·47	6·7	0·131	3·26	0·63	2·72	5·12	·670	
21	1st Feb. 1929	M	1·031	15·32	4·8	0·131	3·62	0·63	2·93	5·98	·796	
22	Do.	E	1·031	13·37	4·9	0·225	3·33	0·65	2·81	5·12	·706	
23	2nd Feb. 1929	M	1·033	13·58	4·3	0·225	3·46	0·60	2·79	5·24	·736	

E

TABLE III

Colostrum analysis (Third Period)

Serial No.	Date and time of sampling		Specific Gravity at 60°F.	Total Solids Per cent	Fat Per cent	Acidity Per cent	Total Proteins Per cent	Albumin and Globulin Per cent	Casein Per cent	Lactose Per cent	Mineral matter Per cent	Remarks
1	16th Feb., 1929	M	1·058	19·98	2·7	3·42	11·84	8·49	3·57	3·67	1·55	M. Morning milk. E. Evening milk.
2	Do.	E	1·044	18·79	5·5	0·288	8·28	3·71	4·33	4·22	1·34	
3	17th Feb. 1929	M	1·038	15·54	4·4	0·216	6·72	3·05	3·45	4·57	1·17	
4	Do.	E	1·034	15·86	5·0	0·221	4·65	1·21	3·57	4·54	0·933	
5	18th Feb. 1929	M	1·034	16·02	5·1	0·198	3·48	0·89	3·35	4·60	1·180	
6	Do.	E	1·034	14·74	4·4	0·207	3·36	1·05	2·39	5·04	0·832	
7	19th Feb. 1929	M	1·034	14·79	4·4	0·203	4·53	0·96	3·08	4·33	0·912	
8	Do.	E	1·033	15·33	5·0	0·230	4·33	0·96	3·39	4·02	0·800	
9	20th Feb. 1929	M	1·035	15·43	5·1	0·203	4·42	1·07	3·55	4·39	0·818	
10	Do.	E	1·032	14·82	4·8	0·176	3·82	0·89	3·08	4·83	0·796	
11	21st Feb. 1929	M	1·032	14·44	4·6	0·221	3·84	0·83	2·92	4·73	0·790	
12	Do.	E	1·032	Spoiled	5·2	0·185	3·73	0·78	2·99	4·63	0·785	
13	22nd Feb. 1929	M	1·032	Do.	4·3	0·176	3·91	0·83	3·15	4·33	0·820	
14	Do.	E	1·032	14·49	4·4	0·149	3·77	0·83	2·43	4·39	0·811	
15	23rd Feb. 1929	M	1·032	14·44	4·5	0·167	3·77	0·85	3·08	4·28	0·791	
16	Do.	E	1·033	14·93	4·8	0·162	3·60	0·78	2·88	4·90	0·802	
17	24th Feb. 1929	M	1·032	15·22	5·2	0·171	3·75	0·85	2·93	5·04	0·799	
18	25th Feb. 1929	M	1·031	13·36	4·6	0·126	3·51	0·83	2·57	4·63	0·785	
19	Do.	E	1·032	13·59	5·2	0·104	3·57	0·72	2·88	4·70	0·776	
20	26th Feb. 1929	M	1·031	14·01	4·8	0·158	3·60	0·76	2·97	4·63	0·761	
21	Do.	E	1·031	13·35	5·2	0·234	3·68	0·80	3·02	3·93	0·768	
22	27th Feb. 1929	M	1·031	13·70	4·7	0·180	3·51	0·76	2·99	3·98	0·759	
23	Do.	E	1·031	13·89	4·9	0·140	3·62	0·76	2·97	4·45	0·779	
24	28th Feb. 1929	M	1·032	14·01	4·7	0·131	3·66	0·72	3·02	4·57	0·771	

TABLE IV

Colostrum analysis (Average of three periods)

Serial No.	Specific Gravity at 60° F	Total Solids Per cent	Fat Per cent	Acidity Per cent	Total Proteins Per cent	Albumin and Globulin Per cent	Casein Per cent	Lactose Per cent	Mineral matter Per cent	Remarks
1	1·058	22·55	4·6	0·368	13·21	8·70	4·58	3·30	1·230	
2	1·042	18·52	6·0	0·266	8·29	4·40	3·94	4·13	1·038	
3	1·036	14·70	3·8	0·230	5·90	2·33	3·32	4·25	1·007	
4	1·033	14·23	4·1	0·230	4·38	1·26	3·03	4·24	0·847	
5	1·032	14·90	5·3	0·209	3·97	1·13	3·13	3·36	1·033	
6	1·031	15·75	5·6	0·206	3·85	1·04	2·78	4·69	0·866	
7	1·032	15·58	5·4	0·194	4·41	0·96	3·32	4·48	0·879	
8	1·032	15·70	5·4	0·196	4·23	1·01	3·33	4·62	0·770	
9	1·033	15·49	5·6	0·198	4·29	0·97	3·36	4·84	0·855	
10	1·032	14·84	5·1	0·191	4·02	0·89	3·25	5·02	0·771	
11	1·032	14·73	4·4	0·197	4·02	0·84	3·09	4·82	0·814	
12	1·030	15·79	5·8	0·173	3·80	0·79	3·01	4·62	0·812	
13	1·031	15·21	5·1	0·173	3·83	0·90	3·05	4·43	0·810	
14	1·031	14·93	5·3	0·180	3·72	0·82	2·77	4·43	0·829	
15	1·031	14·58	5·5	0·189	3·73	0·87	2·97	4·25	0·832	
16	1·031	15·85	5·8	0·204	3·65	0·85	2·88	4·59	0·761	
17	1·031	14·76	5·3	0·195	3·62	0·80	2·93	4·82	0·700	
18	1·031	14·32	4·7	0·164	3·49	0·77	2·69	4·56	0·803	
19	1·032	13·40	4·3	0·162	3·59	0·72	2·80	4·85	0·818	
20	1·030	14·51	5·6	0·159	3·45	0·74	2·86	4·73	0·750	
21	1·031	14·62	5·2	0·179	3·64	0·75	3·00	4·77	0·796	
22	1·031	13·72	4·8	0·192	3·55	0·75	2·95	4·59	0·751	
23	1·032	13·73	4·6	0·182	3·54	0·68	2·88	4·85	0·757	
24	1·032	14·01	4·7	0·131	3·66	0·72	3·02	4·57	0·771	

TABLE V

Description of the sample of Fat from milk	Saponification value	Iodine Value	Refractive Index at 40° C
1st day after calving	203·6	32·31	1·457
2nd Do.	206·4	33·73	1·457
3rd Do.	205·6	33·56	1·457
4th Do.	210·9	30·99	1·456
5th Do.	217·3	31·39	1·456
6th Do.	220·2	31·03	1·456
7th Do.	222·8	32·23	1·455
Normal for butter Fat	224·1	31·0	1·455

BOVINE TRYPANOSOMIASIS IN CENTRAL PROVINCES WITH AN ACCOUNT OF SOME RECENT OUTBREAKS

BY

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(With Plates VIII—X.)

The term " bovine trypanosomiasis " in the title is used advisedly in view of the fact that, as is to be explained later, the form of trypanosomiasis dealt with in this paper presents certain features which would appear to make it necessary to carry out an extensive investigation into this condition before it can be definitely identified with Surra, by which term is understood the type of trypanosomiasis caused exclusively by *Trypanosoma evansi*. One of these features is the frequency with which the disease progresses to a fatal issue for until recently *T. evansi* has been regarded as usually innocuous for cattle.

This point has been mentioned in the Annual Report of the Imperial Institute of Veterinary Research, Muktesar, for the year ending March 1935, where it is stated, " In view of the recent outbreaks of a fatal form of cattle surra at Karnal (Punjab) and Hyderabad (Deccan), work has been taken in hand (1) to determine the specific identity of the trypanosomes concerned in such outbreaks, and if exhaustive researches into the identity of the parasites show them to be identical with *T. evansi*, (2) to ascertain the conditions under which *T. evansi* which is usually innocuous for the bovine species, attains the status of a virulent variant for these animals."

From available literature, it would appear that, the first severe outbreak of cattle trypanosomiasis in India, was reported from Madras by Chetti [1922], according to whom, out of a total of 111 Corporation bullocks affected with the disease, as many as 101 animals died, the symptoms ranging between the acute and the chronic form. About nine years later, Rao [1931], working in the same Province, recorded a series of similar outbreaks with a mortality rate of 20 to 30 per cent.

Quite recently, a very severe outbreak of cattle trypanosomiasis has been reported by Mahajan [1934] from Hyderabad (Deccan), with as high a mortality as 100 per cent, about 90 per cent of the affected animals being bullocks.

In a still more recent communication, Iyer and Sarwar [1935] have reported similar outbreaks of the disease at the Imperial Cattle Breeding Farm, Karnal (Punjab).

In the Central Provinces itself, an outbreak of cattle trypanosomiasis was reported by Stirling [1927] from the Western Districts, and, as is to be described later, the present writer recently came across several outbreaks of the same disease in the Saugor District, with a mortality rate of more than 96 per cent, these observations completely disproving the hypothesis hitherto held that cattle and buffaloes act merely as " reservoirs " of trypanosomiasis without exhibiting any clinical symptoms of the disease.

SOME OUTBREAKS OF THE DISEASE

The first outbreak of the disease reported to the writer was from Kurwai State, from where it spread quickly to the border villages of Khurai *tahsil* of Saugor district, with the result, that within a short period the whole area lying along the border became affected. The author immediately availed himself of the opportunity to study the outbreak, and undertook investigation at Bamora village on the 2nd October, 1935, where the disease was confirmed by microscopical examination in six cases. Altogether five villages were visited in that area and the number of deaths noted in each was approximately as follows :—

Name of village	Bullocks	Cows	Buffaloes	Total
Bamora	6	2	1	9
Satni	5	3	2	10
Padriya	13	4	3	20
Ghamera	4	2	..	6
Total	28	11	6	45

In the course of this investigation, blood from 663 animals was examined in wet smears and out of these 24 cases were confirmed for bovine trypanosomiasis. Of the affected animals, 90 per cent were bullocks and only a small proportion were cows and buffaloes. All these animals were found to be suffering from an acute type of the disease.

The disease spread so widely and spontaneously that from September till the end of October, the Veterinary Assistant Surgeons in charge of Khurai and Saugor dispensaries, attended as many as 30 and 23 outbreaks in their jurisdiction respectively. The disease was confirmed in most of the villages by the Central Laboratory, Nagpur, on examination of blood smears. In these 53 outbreaks a total of 280 animals were reported as having succumbed to the disease, and in all the cases, particularly in bovine species, it was of an acute and virulent type. It is most likely that more villages situated in the interior were likewise affected, although no statistics regarding the mortality caused in these localities are available.
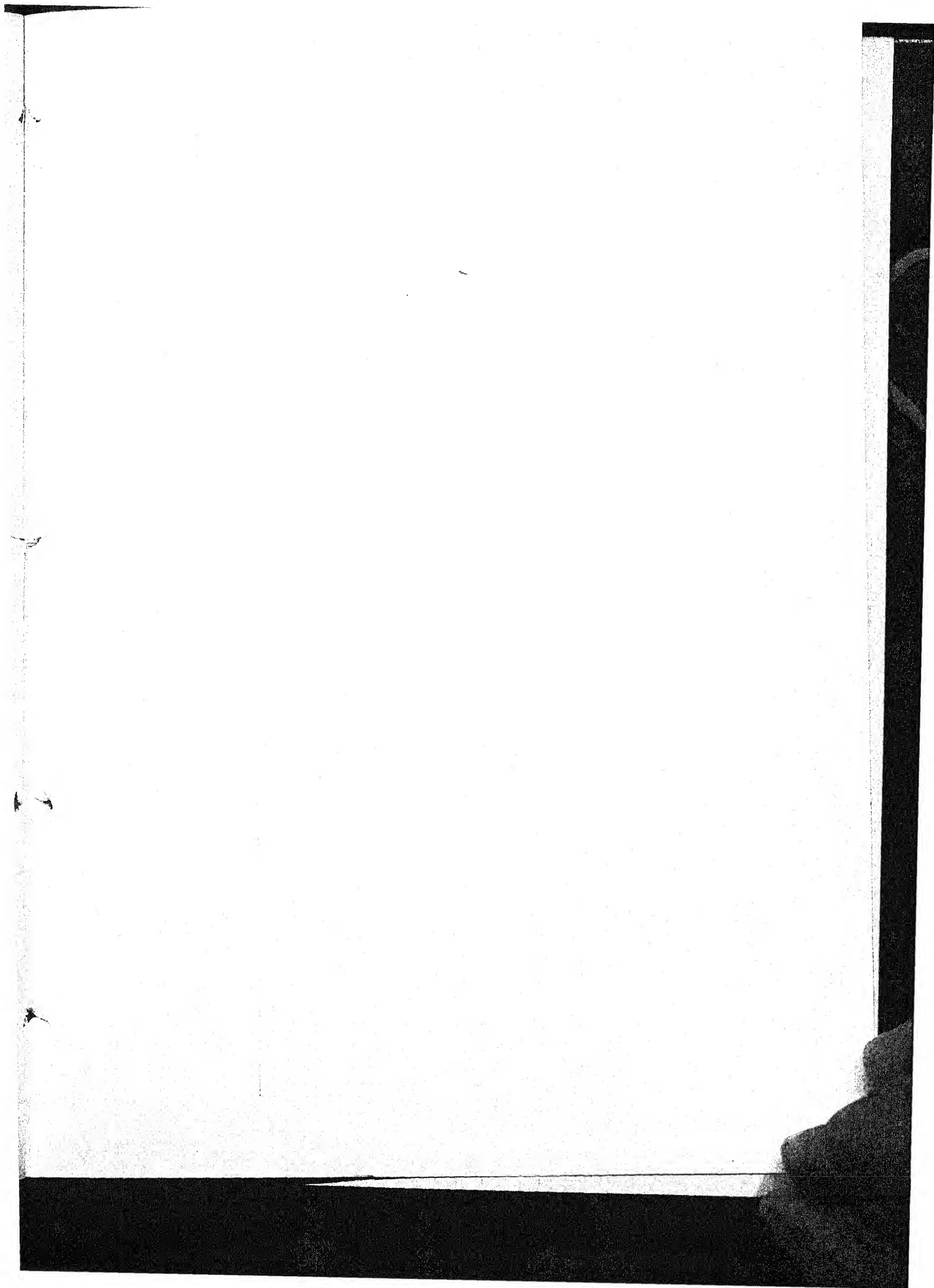
FIG. 1.   Bullock No. 2—An acute case in initial stage.

FIG. 2.   Cow No. 18—Fatal Stage after an acute course.
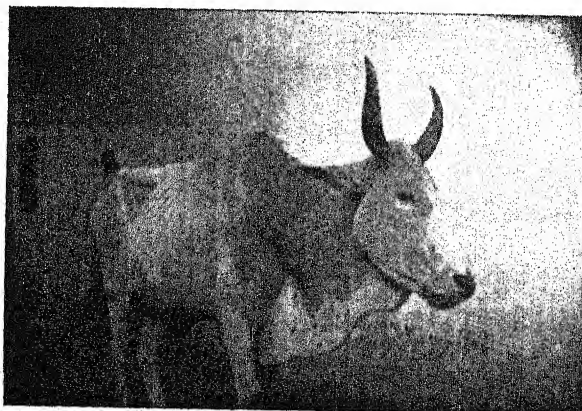
FIG. 3.   Bullock No. 27—A chronic case with impaired vision.

The second interesting outbreak of bovine trypanosomiasis encountered by the author occurred in Damoh *tahsil* in Saugor district, towards the end of November. The author's attention was at first directed on perusal of the contagious disease register to the record of certain outbreaks, which had passed either for Anthrax or for *bhora*, but no definite diagnosis seemed to have been made in these cases by the local veterinary staff. About the same time another similar report of *bhora* came from a village known as Mala. On the 24th November, this village was visited by the author and there he found a heifer *in extremis,* and its blood revealed the presence of trypanosomes under the microscope.

During the course of investigation, the local cultivators told the writer that the disease was also prevalent in the adjacent villages of Rond and Sagra. On receipt of this information an investigation was undertaken on the 26th and 27th November in both these villages, where the disease proved to be mostly of a chronic type, with a high rate of mortality.

The actual number of deaths recorded up to the 26th October in these two localities were as follows :—

Name of village	Bullocks	Cows	Young calves	Buffaloes	Buffalo calves	Ponies	Total
Rond . . .	26	21	4	6	6	1	64
Sagra . . .	16	12	11	1	5	5	50

The number of cases found suffering from the disease at the time of the author's first visit was as follows :—

Name of village	Bullocks	Cows	Young calves	Buffaloes	Buffalo calves	Ponies	Total
Rond . . .	10	11	2	2	..	..	25
Sagra . . .	16	4	4	..	..	..	24

The disease was confirmed microscopically in ten animals at both these villages, and in all the suffering cases, the method of treatment adopted was an intravenous administration of tartar emetic. Both the villages were visited again on the 11th December when the disease was found to be still prevailing, more than thirty fresh cases having occurred during the interval. Out of fifty cases treated with tartar emetic during the author's previous visit, thirty-five were reported to have succumbed and the rest recovered.

In the course of this investigation, it was observed, that the disease ran an acute, sub-acute and also a chronic course (Plate VIII, Figs. 1—3 and Plate IX, Fig. 4) with the usual characteristic symptoms. As the symptoms have already been described by the different workers in this country, it seems unnecessary to recapitulate them in this article.

Microscopic examination of blood reveals the presence of trypanosomes in the acute form of the disease quite readily, whereas in the sub-acute and chronic forms they are rarely seen in the peripheral circulation. Soon after death, smears of heart blood, lung, liver and cerebro-spinal fluid failed to reveal any trypanosomes.

SUSCEPTIBILITY

Bullocks when overworked proved to be more susceptible than other cattle, while on the other hand buffaloes appeared to resist the disease. Some young calves below six months of age were reported to have succumbed during the recent outbreaks, and in the case of one calf under six months, the author detected trypanosomes on examination of blood smears.

REGIONAL DISTRIBUTION

A map is appended (Plate X) to indicate the incidence of bovine trypanosomiasis as observed during the current year, the disease in all cases having been confirmed microscopically on examination of blood smears in the Veterinary Laboratory, Nagpur. In Saugor district, where the author investigated the problem, the area chiefly affected was low lying and covered with a wild growth of tall thick grass known as *kans*, a large part of the area remaining under water during rainy season.

SEASONAL INCIDENCE

The disease runs a definite seasonal course. The first outbreaks occurred during the month of August, and these spread extensively during September, October and November, when they began to subside. Some chronic cases occurred in December and January in the villages where the disease had already been prevailing since the month of November, but no fresh outbreak was reported during the later two months.

TREATMENT

Twenty-one animals were treated in the month of October by means of tartar emetic solution administered intravenously, the dose used varying from 10 to 15 grains in 20 to 30 c.c. of distilled water. All the twenty-one animals were suffering from an acute type of the disease. This method of treatment proved to be strikingly successful, for only one of the treated animals succumbed and all others recovered. The efficacy of this method of treatment was also tested upon more than fifty chronic cases, the majority of which were in an advanced stage of the disease, but the results were not so encouraging, for the treatment was successful in about 30 per cent of the cases only. These results are summarized in Tables I & II.
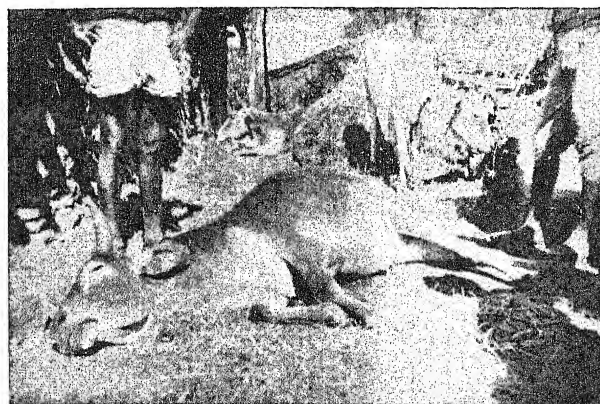
FIG. 1.   Bullock No. 24—Fatal stage after a chronic course. The photograph shows two other affected animals.

FIG. 2.   Bullock No. 7—Indicating sloughing of parts due to firing in an affected case.

Ind.

PLATE X.

CENTRAL PROVINCES & BERAR.

Scale 1 Inch = 64 Miles.
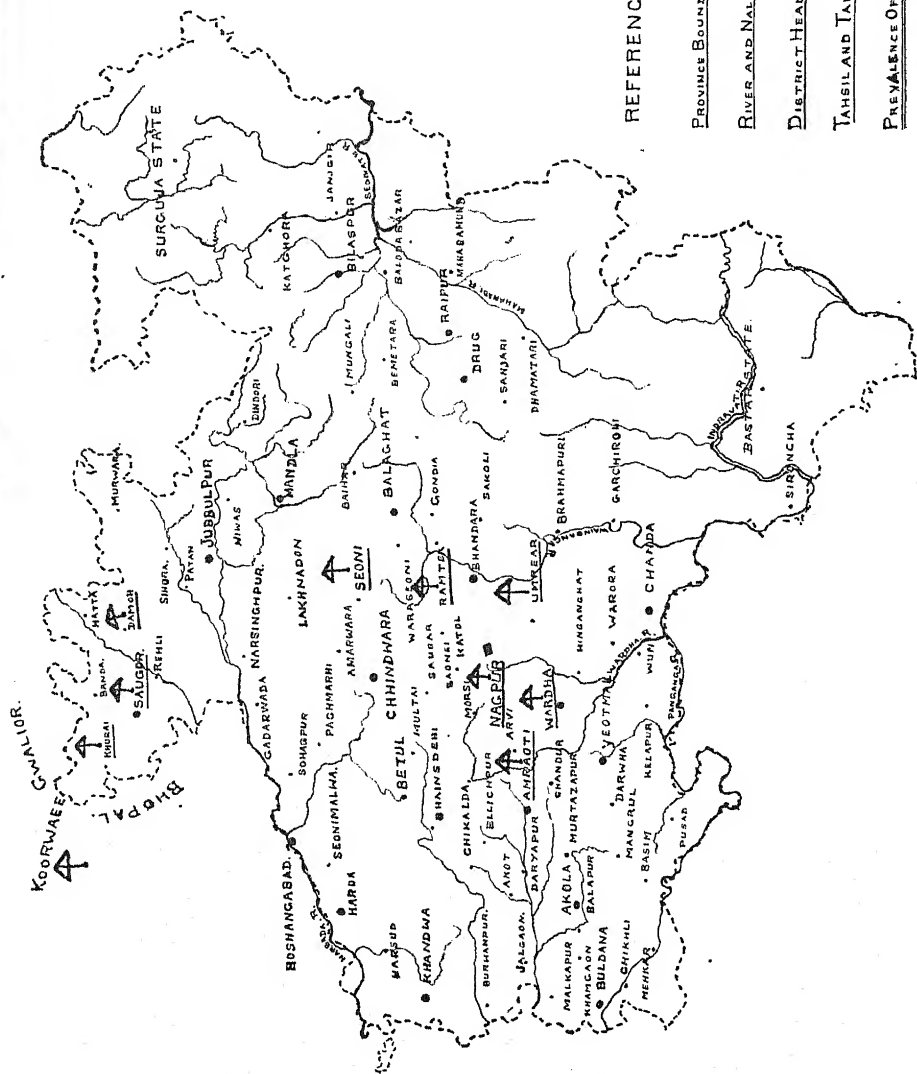
REFERENCES:—

Province Boundary..............
River and Nala.............
District Head Quarters...........
Tahsil and Taluk Head Quarters.....
Prevalence Of Bovine Trypanosomiasis.

During The Year 1935.

Map showing prevalence of trypanosomiasis in Central Provinces.

Ind.

TABLE I

Table showing the result of treatment with tartar emetic given intravenously during initial stage in acute form of the disease

Village	Animal	Symptoms	Blood examination	Date of treatment	Date of second visit	Blood examination	Remarks
Bamora, P. C. 39	Bullock No. 1	Off feed, lachrymation dullness.	Positive for trypanosomiasis.	3rd Oct, 1935	7th Oct., 1935	Negative	Animal recovered, symptoms subsided.
	Bullock No. 2	Initial stage	..	..	Do.	Do.	Recovered.
	Bullock No. 3	Do.	Negative	Do.	Do.	Do.	Do.
	Bullock No. 4	Do.	Do.	Do.	Do.	Do.	Do.
	Bullock No. 5	Do.	Do.	Do.	Do.	Do.	Do.
	Bullock No. 6	Do.	Positive	Do.	Do.	Do.	Do.
	Bullock No. 7	Do.	Negative	Do.	Do.	Do.	Do.
	Bullock No. 8	Do.	Do.	Do.	Do.	Do.	Do.
	Cow No. 9	Do.	Do.	Do.	Do.	Do.	Do.
	Bullock No. 10	Do.	Positive	Do.	Do.	Do.	Symptoms subsided, animal fully recovered.
	Bullock No. 11	Do.	Do.	7th Oct., 1935	25th Nov., 1935	Do.	Do.
Padriya	Bullock No. 12	Do.	Do.	Do.	Do.	Do.	Do.
	Buffalo No. 13	Latent stage.	Do.	2nd Oct., 1935 (2 injections 15 grains each.)	The animal died on the 9th October, 1935.	..	Died.
	Bullock No. 14	Initial stage	Do.	7th Oct., 1935	25th Nov., 1935	Negative	Recovered.
	Bullock No. 15	Do.	Do.	Do.	Do.	Do.	Do.
	Bullock No. 16	Do.	Do.	Do.	Do.	Do.	Do.
	Bullock No. 17	Do.	Do.	Do.	Do.	Do.	Do.
	Cow No. 18	Do.	Do.	Do.	Do.	Do.	Do.
	Cow No. 19	Do.	Do.	Do.	Do.	Do.	Do.
	Heifer No. 20	Do.	Do.	Do.	Do.	Do.	Do.
	Bullock No. 21	Do.	Do.	Do.	Do.	Do.	Do.
	Bullock No. 22	Do.	Do.	Do.	Do.	Do.	Do.
	Total No. 22	..	..	Death 1		..	Recoveries 95 per cent.

F

TABLE II

Table showing the results of treatment in chronic form of the disease

Village	Animal	Stage of disease	Blood examination	Date of treatment	Date of 2nd visit	Remarks
Mala, Damoh tahsil.	Cow No. 23	Latent stage	Positive	23rd Nov., 1935	25th Nov., 1935	Animal died on 24th November, 1935.
	Bullock No. 24	Do.	Negative	27th Nov., 1935	11th Dec., 1935	Died.
	Bullock No. 25	Do.	Do.	Do.	Do.	Do.
	Bullock No. 26	Do.	Do.	Do.	Do.	Do.
	Bullock No. 27	Do.	Positive	Do.	Do.	Do.
	Bullock No. 28	Do.	Negative	Do.	Do.	Do.
	Bullock No. 29	Do.	Do.	Do.	Do.	Do.
	Buffalo No. 30	Do.	Do.	Do.	Do.	Do.
	Buffalo No. 31	Do.	Do.	Do.	Do.	Do.
	Buffalo No. 32	Do.	Do.	Do.	Do.	Do.
	Cow No. 33	Do.	Positive	Do.	Do.	Do.
	Cow No. 34	Do.	Do.	Do.	Do.	Do.
	Cow No. 35	Do.	Do.	Do.	Do.	Do.
	Cow No. 36	Do.	Do.	Do.	Do.	Do.
	Cow calf No. 37	Do.	Negative	Do.	Do.	Do.
	Cow calf No. 38	Sub-acute	Positive	Do.	Do.	Recovered.
	Bullock No. 39	Do.	Negative	Do.	Do.	Do.
	Bullock No. 40	Do.	Do.	Do.	Do.	Do.
	Bullock No. 41	Do.	Do.	Do.	Do.	Do.
	Bullock No. 42	Do.	Do.	Do.	Do.	Do.
	Cow No. 43	Do.	Do.	Do.	Do.	Do.
	Cow No. 44	Do.	Do.	Do.	Do.	Do.
	Cow No. 45	Do.	Positive	Do.	Do.	Do.
	Cow No. 46	Do.	Do.	Do.	Do.	Do.
	Cow calf No. 47	Do.	Do.	Do.	Do.	Died.
	Cow calf No. 48	Do.	Do.	Do.	Do.	Do.

Total 26.   Deaths 18.   Recoveries 8.

IDENTITY OF THE CAUSAL ORGANISMS

A number of blood smears were submitted to the Muktesar Laboratory for examination and the following report was received :—

"The blood smears forwarded have, on microscopical examination, shown *Trypanosoma evansi* in each case ".

IDENTITY OF TICKS AND FLIES

Specimens of flies and ticks, collected from some of the affected animals, were forwarded for examination to the Muktesar laboratory. The flies were identified as *Hippobosca maculata* and the ticks as *Hyloma aegyptium* and *Boophilus australis.* Blood smears, made from some of the crushed ticks, proved negative for the presence of trypanosomes.

It is of interest that the disease is known as *bhora* amongst the local cultivators, the name being based upon the phenomenon of circular movements manifested by the affected animals. The disease is believed by some to be brought about as a result of ingesting certain species of worms found on the long grass, while others believe it to be caused by snake bite. Firing is the only treatment commonly practised by the local cultivators, and it often leads to sloughing of the parts treated in this manner (Plate IX, Fig. 5).

SUMMARY

1. Although bovine trypanosomiasis is not of infrequent occurrence, the disease has hitherto been mistaken for anthrax in certain tracts in the province.

2. The disease affects both cattle and buffaloes but working bullocks are particularly susceptible to it, the rate of mortality in these, when affected with the acute form of the disease, being more than 90 per cent. Young cattle come second in regard to susceptibility, while buffaloes are more resistant.

3. Some cases of death also occurred among young calves below six months of age, and in one instance the trypanosomes were detected under the microscope.

4. The disease runs an acute, sub-acute and chronic course. In the acute form, the death may occur from six hours to twenty-four hours from the onset of symptoms. In the chronic type, the animal may linger as long as one to three weeks.

5. The disease has a definite seasonal incidence. It appears soon after the rains and continues till the latter part of the winter.

6. The disease prevails particularly in low-lying areas inundated during the rains and covered with a wild growth of tall, thick grass known as *kans.*

7. During 1935, very serious outbreaks of the disease have been recorded in Saugor district, with a mortality rate of more than 96 per cent.

8. The trypanosomes concerned in these outbreaks were examined at the Muktesar laboratory and found indistinguishable from *Trypanosoma evansi.*

9. The method of treatment adopted was an intravenous injection of tartar emetic and this proved very effective when given in the initial stage of an acute form of the disease. In the chronic form, however, it proved successful only in 30 per cent of the cases.

ACKNOWLEDGMENTS

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AN UNUSUAL CASE OF EPIZOOTIC LYMPHANGITIS IN A MULE

BY

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(With Plates XI—XIII.)

Epizootic lymphangitis is a virulent disease of equines, caused by the specific fungus, *Cryptococcus farciminosus* Rivolta, and it has no doubt existed from time immemorial in many parts of the world where it has often been confounded with cutaneous glanders or ulcerative lymphangitis. French veterinarians have, however, recognized the disease as a separate entity for a long time under such names as *Farcin de riviere, Farcin en cul de poule, Farcin curable, Farcin d' afrique* and *Lymphangite Epizootica.*

Epizootic lymphangitis is usually considered as a suppurative disease of the lymphatic vessels and glands of the skin only, and the possibility of the occurrence of atypical cases affecting the internal organs, with the total freedom of the skin, is, we believe, often overlooked. The diagnosis of such atypical cases presents obvious difficulties. For the above reason and since a consideration of these atypical forms of Cryptococcosis is likely to throw some light on the probable modes of natural transmission of the disease, about which there does not appear to be any unanimity of opinion, it is proposed to record in this article the case of a mule which succumbed to an infection of the intestine, the usual cutaneous lesions being entirely absent.

LITERATURE

In order that the present case may be studied in its proper perspective, it would seem worth while to notice briefly and in chronological order some records of epizootic lymphangitis involving sites other than the skin.

Nocard [1891] pointed out the constant association of the parasite with the lesions and how easily a diagnosis can be made by demonstrating their presence. He called attention to the occurrence of lesions on the mucous membranes in some animals resembling those of glanders.

Mazzanti [1892] found, on *post mortem* examination of a mare $2\frac{1}{2}$ years old, which died showing symptoms of enteritis, certain lesions in the terminal part of the colon. The colon showed an abscess of the size of a pigeon's egg, and in the vicinity the mucous membrane presented one irregular ulcerated area, about 12 cm. in diameter, with raised borders, a depressed centre and covered with

black alimentary debris. On histological examination, the indurated wall of this ulcer revealed suppurating areas, with cryptococci located in the cells and outside them. This reference constitutes the only record of the occurrence of intestinal cryptococcosis in equines, excepting perhaps the cases of Cornish Bowden [1904] and Descazeaux [1921] to be mentioned below.

Nocard and LeClainche [1896] state that the specific lesions may extend to the larger bronchi and that occasionally they are found in the lung parenchyma as well.

In Japan the condition had been known for years under the name " Japanese Farcy " and Tokishige [1896] claims to have found it occurring in cattle besides horses. He observes that in equines the lesions may be found in the lungs, liver, spleen and bones, and that the lung lesions occur in the form of lobular pneumonia with fairly frequent greyish nodules resembling those of glanders. It should be noted here that Tokishige was the first worker to cultivate this organism and to allocate it to the class *Saccharomyces*.

In India, this condition was first described by Moore [1896] under the name ulcerative lymphangitis. In giving a detailed account of Moore's cases, Pease [1897] also regarded them as identical with ulcerative lymphangitis. Lingard [1901], the first worker to recognise the organisms in this country, was of the opinion that the outbreaks of this condition at Karnal and Hapur originated from some mules imported from Italy. With a view to determine the relative susceptibility of each species he carried out, by the subcutaneous route, a series of experimental inoculations on equines, bovines, buffaloes, sheep and goats with fresh material from a clinical case. A few weeks after the inoculation, large lesions were observed at and around the seat of inoculation in the horses and mules, and to a smaller extent in bovines. None of the animals, however, was found to have developed any internal lesions, when autopsied about two years later.

Pallin [1904], in his excellent treatise on this disease, states that usually the lesions of epizootic lymphangitis are confined to the skin, but occasionally they may occur on the mucous membranes and they may even extend to the internal organs. The lesions may be found on any part of the body, but are frequently associated with parts exposed to wounds from kicks, contusions and harness galls. This worker further observes that in about 7 to 10 per cent of cases, the mucous membrane covering the nasal chambers, air sinuses, the pharynx, larynx and the upper third of the trachea and also the conjunctiva are affected. The mucosal lesions, it is stated, are first noticed as small papules or pimples which rapidly develop into vesicles and burst, forming well-defined ulcers with a raised edge and a dug out centre. Pallin succeeded in proving that the disease is readily inoculable to horses, donkeys and mules, but he failed to reproduce the disease in cattle, sheep, goats and guinea-pigs by inoculation. According to him, systemic disturbances rarely ensue in the early stages of the cutaneous form ; but in advanced stages intermittent fever and a rapid fall in condition have been observed.

Working in Great Britain, Runciman [1904] observed that the first symptoms of the disease are a slight rise of temperature and sometimes a filling of the hind limbs, diminished appetite and a rapid loss of condition. No further change takes place for days, until suddenly cutaneous nodules make their appearance and cover the whole of the animal's body within a space of 12 to 24 hours. Such cases are not amenable to any treatment and rapidly progress to a fatal issue. Until the appearance of the nodules, the symptoms observed are extremely vague and provide little of diagnostic value. According to this worker, cases of this kind are of digestive or respiratory origin, the primary nodules being present in the internal organs. In the primary cutaneous form of the disease, he is unaware of any symptoms other than the presence of the nodules ; while he is confident that cases of primary internal infection do occur where the condition can be diagnosed only after the appearance of the specific nodules in a visible part of the body. He cites a few cases of primary internal infection he encountered, where the animals were sick a long time before the actual appearance of external lesions. In one of his cases, where the animal was ill for some time, the nodules appeared all of a sudden and the animal died. On *post mortem* examination, abscesses were found in the lungs which contained a thick creamy pus, suggesting to him that the lungs were the primary seat of infection. Hence Runciman considers it as hardly probable that the infection in this disease is conveyed exclusively through the skin.

Cornish Bowden [1904] records the particulars of a horse exhibiting severe premonitory and systemic symptoms which were followed subsequently by severe diarrhoea and cutaneous lesions. This animal was purchased in good condition on 7th July, 1904, it went off feed a week later, and rapidly went down in condition until it developed severe foetid diarrhoea, with small cutaneous sores which were noticed later on 20th July, 1904. The animal, however, was successfully treated with potassium iodide, iodine and quinine and was discharged, cured on 18th August, 1904. This was presumably a case of the disease where infection of the intestines preceded the involvement of the skin.

Harber [1913] had experience of numerous well marked lesions appearing on the body of horses in the course of 48 hours, and of some unbroken buds appearing in 24 hours. However, he was unable to detect any wounds on the affected animals, which might have served as portals of entry for the parasites concerned. He remarks that, in these cases, the organisms could not have been carried by harness, etc., as these animals were kept in strict quarantine and were not being worked. Harber [1913] presumes that the disease becomes generalized to the cutis from primary lesions in the internal organs, although he failed to encounter cases of this kind personally. He adds that the disease spreads more rapidly in donkeys than in horses, and concludes that flies are mainly involved in the spread of the infection.

Tokishige's contention that cattle in Japan may develop atypical lesions of the disease has been questioned by Wallis Hoare [1913], since this view is not

in accord with the fact that bovines in other countries have resisted all attempts at experimental infection. Hoare goes on to say that the disease being so prevalent in Japan, almost every horse is liable to develop an attack at an early age, and that a horse is considered more valuable there after recovery than if it had never had an attack, as it is then held to be immune.

In Morocco, Menod and Velu [1915] described what they believe to be cases of delayed relapses among horses, as opposed to reinfection, occurring from 3 to 6 months after apparent complete recovery. These workers refer to the occurrence of osseous lesions in this disease, averring at the same time that these constitute the most serious and refractory form. They add that, with rare exceptions, the extracutaneous lesions are often secondary, and that out of 20 cases of epizootic lymphangitis slaughtered as incurable in one year, 13 were found to have developed bony lesions.

Fayet, Leysses and Prudhomme [1917] record under the heading " a rare seat of epizootic lymphangitis " lesions in the post-pharyngeal lymphatic glands of a horse.

In the experience of Descazeaux (1921], a horse, which was under treatment for epizootic lymphangitis with lesions about the withers, died of some digestive disorder. In this case, cryptococci were found in the enlarged prescapular and axillary lymph glands, but the stomach and intestines showed no specific alteration whilst the mesenteric glands appeared healthy. However, smears from the mucous membrane of the stomach and intestines, as also from the contents of a *Habronema* abscess in the stomach showed on examination the presence of numerous cryptococci. Histological examination of the *Habronema* tumour showed masses of cryptococci and the helminths, whilst giant cells containing numerous cryptococci were found in the submucous lymph spaces.

Velu [1924] proposes the name *Cryptococcus marandei* for the parasite affecting the lachrymal tract of donkeys, and adds that the parasite can be transmitted readily from donkey to donkey with infective material and that filamentous forms and ascospores do not occur in these parasites.

Gronow [1924] found mycelial and yeast forms of the parasite in the pus from a local abscess of a horse produced by subcutaneous inoculation of a living culture of the organism.

Michelon [1925] mentions the occurrence of four abscesses in the lungs and one in the bronchial gland of a horse due to *Cryptococcus farciminosus*. The abscesses contained yellowish white pus.

Hutyra and Marek [1926] write that the parasites may also be present in apparently normal lymph glands and lymph spaces of the gastro-intestinal mucous membrane of affected horses, and quote the above-cited experience of Descazeaux in this connection. These workers add that sometimes small nodules and purulent foci may be present in the lungs and testicles of affected horses.

Paine [1931] observes that the disease is more prevalent in tick-infested areas and that ticks take an active part in its transmission.

Bardelli and Cilli [1931] describe a case of osteomyelitis of the left tibia of a horse due to *Cryptococcus farciminosus*. In the interior of the tibial crest, there was a purulent centre, 2 cm. in dimension, surrounded by a zone of reaction manifested as a condensing ostitis, about 5 mm. in width. Numerous cryptococci were seen in the purulent fluid.

From the Sudan, Bennett [1931] describes a form of interstitial pneumonia affecting horses and mules but not donkeys caused by *Cryptococcus farciminosus* and thinks that these lesions may have been overlooked in the past. The symptoms consisted of accelerated and increasingly shallow respiration, irregular rise in temperature, steady loss of condition though the appetite for the most part remained normal. The pleura and the bronchial lymph glands were un-involved, and only in one of the many cases observed was there an evidence of the presence of superficial or cutaneous lesions of epizootic lymphangitis. No other internal organs were affected. From a histopathological point of view, the author writes that the lesions commence with lymphocytic infiltration of the terminal bronchi without any evidence of vascular congestion, the infiltration later on extending to the interlobular septa until one or more whole lobules are involved. The lymphocytes then get replaced or are outnumbered by large mono-nuclear cells derived from the alveolar epithelium and these gradually invade and des-troy the small bronchioles, veins and arteries. This is followed by a fusion of the mono-nuclear cells resulting in the formation of giant cells containing large num-bers of cryptococci, this in turn being followed by the destruction of the invaded cells and the continued proliferation of the parasites. In a later publication Bennett [1932], places on record the occurrence of hyphae of *Cryptococcus far-ciminosus* in a natural case of epizootic lymphangitis. The subject was an aged horse which had extensive lesions, covering practically the whole of its skin, extending to the nostrils and affecting the sub-maxillary lymph glands. In the lungs there was present a number of pea-like scattered nodules, closely simulat-ing glanders. In the smears from the lung nodules, characteristic hyphae of the cryptococcus were encountered, such as are formed in artificial cultures.

History of the Present Case

The clinical material on which this paper is based, was made available to this Institute through the courtesy of Major A. J. Kelly, R.A.V.C., Officer Commanding, Military Veterinary Hospital, Jubbulpore, and at the outset the writer wishes to express his indebtedness to this officer for kind permission to utilise the material for the preparation of this article.

Mule No. 122 of No. 28 A. T. Company was admitted to hospital on 12th November, 1934 in fair condition, but with the visible mucous membranes very

anaemic. Helminthiasis was suspected but the faeces failed to show any worm ova while the blood count was found to be normal. Diarrhoea started on 1st December, 1934 and it persisted for a week, when the mule began to lose condition. Diarrhoea started again on 15th December, 1934 and persisted throughout the progress of the disease. The mule grew weaker day by day, being finally unable to rise. It was destroyed on 3rd January, 1935 by which time it had been reduced practically to a skeleton. In the hospital the animal had received the routine treatment against worms as adopted in Military Veterinary Hospitals in India.

On *post mortem* examination, a few strongyles were found in the intestines. Portions of the bowels and a few mesenteric glands were sent to this Institute for examination. On enquiry, the information was elicited that the mule had shown no cutaneous lesions, nor any wounds or any cording of the lymphatics on the skin. However, it had a history of a punctured foot and of a few days' treatment in hospital about two months prior to its re-admission.

LABORATORY EXAMINATION

Macroscopical examination.—The specimen of the intestines, showed a few well-defined, circular ulcers, with swollen, prominent edges and a dug-out crater-like ragged surface. These ulcers measured from 0·5 to 3·0 cm. in diameter (Plate XI, Fig. I). A few small nodular abscesses containing cheesy purulent material, were also seen on the mucosa, and on pressure some of these discharged a thin purulent material through a minute central hole. The mesenteric glands were distinctly enlarged, and were soft and elastic to feel. When cut into, the entire gland parenchyma was found converted into a whitish suppurating mass (Plate XI, Fig. 2).

Microscopical examination.—Smears from the purulent material from abscesses and from the surfaces of ulcers, when stained by Gram's and Claudius' method, revealed the presence of innumerable *Cryptococcus farciminosus* (Plate XI, Fig. 3). Direct wet smears of pus from the lesions, revealed on examination under a cover-glass the refractile double contoured outlines of the parasites (Plate XII, Fig. 4). The parasites had a slight ovoid body, one end being rounded while the other was generally pointed and measured 3 to 4 microns in length. In stained preparations, some of the parasites were found collapsed and semilunar in appearance, due to the evacuation of the contained body fluid and presumably representing the remains of dead organisms. Some parasites were found to have taken the stain along the periphery only, leaving the central part unstained, while others were completely stained. In a few cases, the cell contents were found to be massed at one end of the cell leaving the other parts clear. Like all other yeast cells,

FIG. 1.   Pieces of intestines showing ulcers on the mucosa.
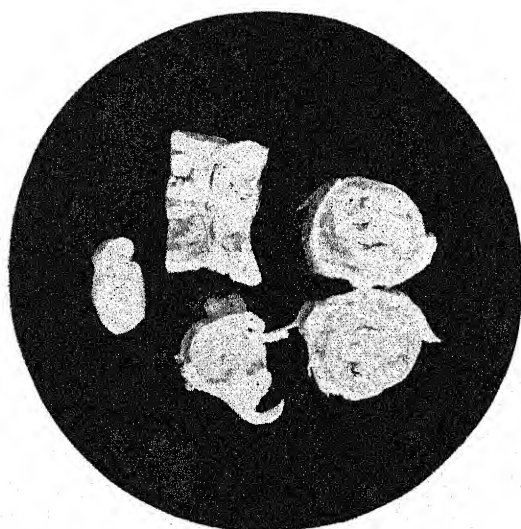
FIG. 2.   Affected mesenteric glands showing cheese-like surface.
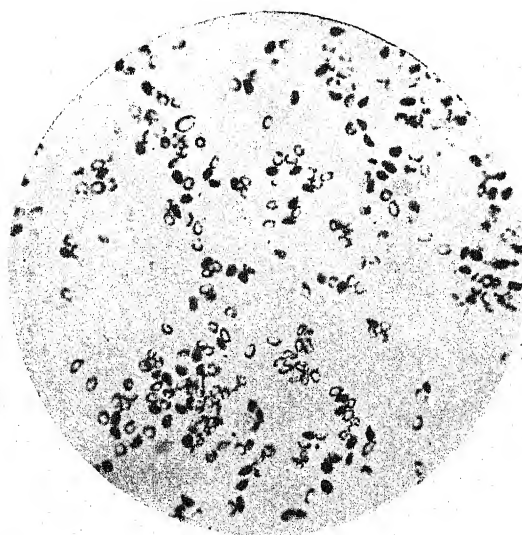
FIG. 3.   Smear from the lesions showing *Cryptococcus farciminosus.* × 800. (Stained Claudius).
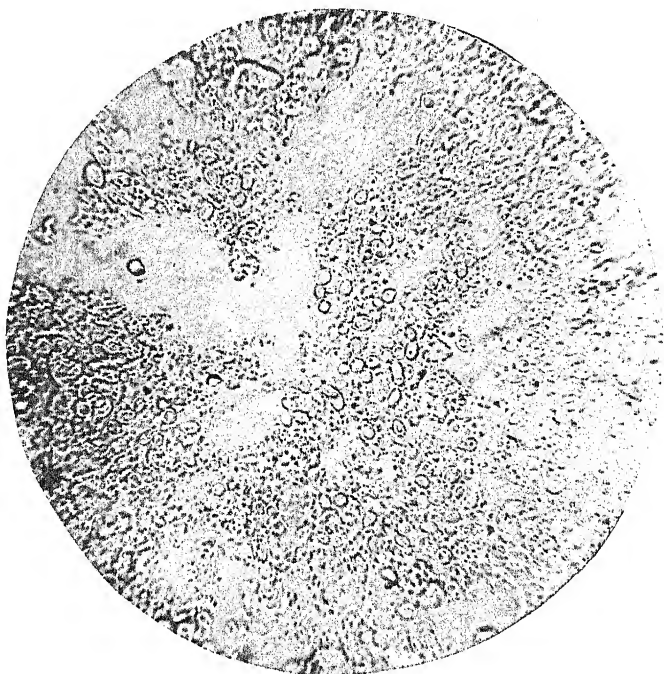
FIG. 4.   Unstained wet pus smear showing the double contoured outlines of
the parasites ×1000.
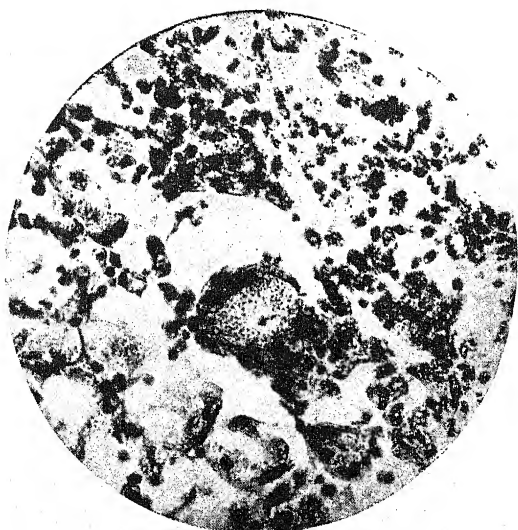
FIG. 5.   Sections of mesenteric gland showing giant cells containing numerous
Cryptococci.   (H.E.)×600.
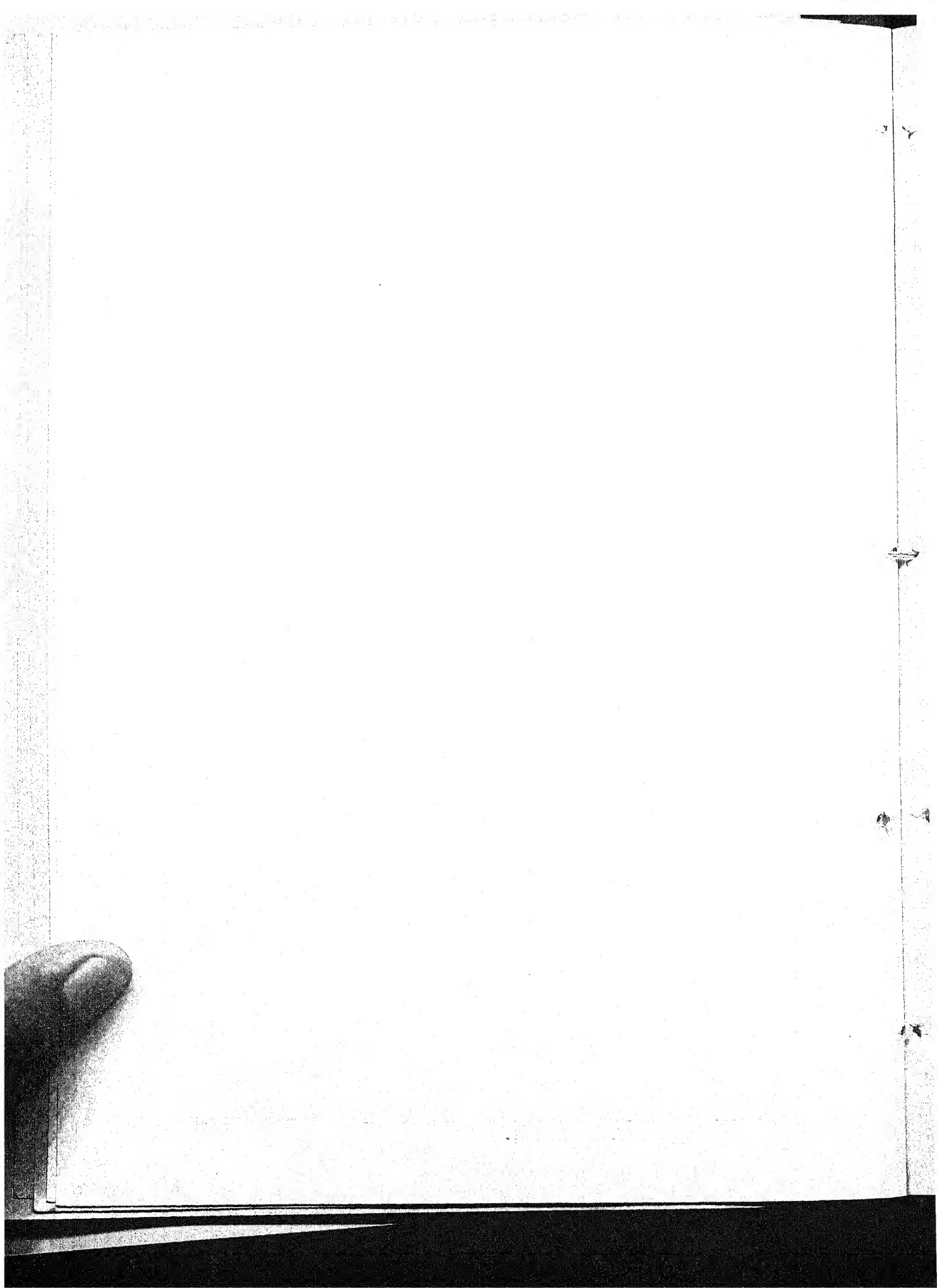
the parasites multiply by a process of budding and such division forms were not infrequent in the smears.

Histopathology.—The lesion commences in the lymph follicles of the intestines where the cells are found with ingested parasites ; these latter then multiply and bring about the rupture of the host cells. The parasites so liberated are in turn phagocytosed or taken up by other cells which also share the same fate. In this way an abscess is formed, surrounded by numerous large mononuclear cells tending to form giant cells. Thus giant cells in various stages of formation are found around the central mass, which consists mainly of parasites and debris of degenerated lymphocytes. The organisms are found in great abundance in the affected tissues both in intra and extra-cellular positions, and their faint outlines are discernible even in sections stained by haemotoxylin and eosin (Plate XII, Fig. 5 and Plate XIII, Fig. 6). Individual cells are often loaded with as many as 10 to 30 parasites and in consequence they are doubled or trebled in their normal size. A group of parasites in a cell often presents an external form suggestive of a mulberry. After the abscess has burst, the breaking down process still continues at the borders and in this way the ulcer gets larger and deeper and new nodules continue to form at the periphery.

One remarkable feature in this condition is the entire absence of fibrous tissue formation around the lesions, which might have limited their extension to the neighbouring tissues. The lesions wherever they occur are structurally the same. The invaded tissues react by a proliferation of the connective tissue cells which tend to form giant cells and ingest the parasites, and an inflammatory abscess is thus formed. Three zones are recognizable in these lesions. The central zone consists entirely of parasites and the debris of broken down cells, the intermediate zone of mononuclear cells, and the outer zone of large endothelial cells and giant cells. In the central zone of the affected lymph glands the parasites are found embedded in an amorphous matrix. In sections stained with haematoxylin and eosin, this central zone presents the appearance of a caseating lesion with no evidence of cellular or nuclear elements. However, when stained for organisms, large masses of cryptococci are seen in this area. The parasites in the older abscesses are found to have lost their staining properties considerably. The pus-like material in these abscesses is formed by the death of the host cells and their subsequent softening, but no actual pus cells have been seen in the smears from this material. The giant cell reaction in the lesions would appear to be one of foreign body reaction, and in the absence of pyogenic organisms and pus cells the extensive necrosis and suppuration in the centre of the lesions are to be ascribed to the action of the cryptococci. The parasites liberated in the evacuations of these intestinal ulcers are passed along the gut when they are phagocytosed and ingested by the cells in the other parts. Extension of the disease to distal portions of the intestines is thus produced. The histological picture, in short, resembles caseating tuberculous lesions, though caseation is not a feature of equine tuberculosis.

MYCELIAL FORMS

The occurrence of mycelial forms of the cryptococcus in pus smears from the lesions of epizootic lymphangitis has been recorded by Tokishige, Gronow and Bennett. In the case now under report, the affected lymph glands revealed pure yeast forms only but in sections of the intestinal ulcers, short and long mycelial forms were seen (Plate XIII, Fig. 7). The entire absence of any other micro-organisms in these sections precludes the possibility of these mycelial forms being contaminants. On the other hand, the absence of mycelial forms in the closed lesions of the lymphatic glands and their presence in the open ulcers of the intestines only suggests that the relative oxygen supply may have been a factor in the production of the mycelia. Though the statement of Castellani [1926], that the hyphae of the members of the family *Cryptococcaceae* are little different from their conidia in appearance, both being yeast-like in form suggests otherwise, long, delicate and filamentous mycelia have been seen in sections in the present instance.

CULTURE

Although the morbid material was sent preserved in 10 per cent formalin, cultures were attempted from the central portion of the larger-sized mesenteric glands, to ascertain if the formalin had not penetrated into this area, but as was expected no growth was obtained and the cultural characteristics of the fungus could not be studied.

DISCUSSION

Although a good deal has been written on the subject of epizootic lymphangitis, there is still no clear evidence as to the mode of infection and the mechanism of the spread of this disease. In the past, attention has been almost entirely confined to the more frequent cutaneous form of the disease, and the current belief has been that the infection takes place through some pre-existing wound in the body. Infection of the mouth and lips has been said to occur as a result of an affected animal biting at ulcers on the legs or other parts of the body.

It would appear certain from the observations recorded in this paper and from those in one or two other records that there are alternative modes of infection as well. For instance in the pulmonary form recorded by Bennett and others, inhalation has been proved to be responsible for the infection ; whereas the already cited experience of Descazeaux, who found cryptococci in a *Habronema* abscess in the stomach of a horse suffering from digestive disorders and dying of epizootic lymphangitis, the oral route must have been responsible.

Similarly in our Jubbulpore case, ingestion was evidently the mode of infection. The worms found on *post mortem* examination in the bowels might have produced some injuries in the mucosa. The ingested Cryptococci, during the
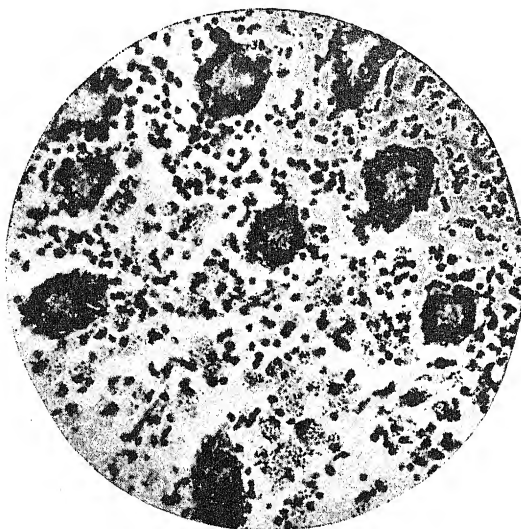
FIG. 6. Sections of intestines showing numerous intracellular cryptococci seen as faint granules. (H.E.) ×500.
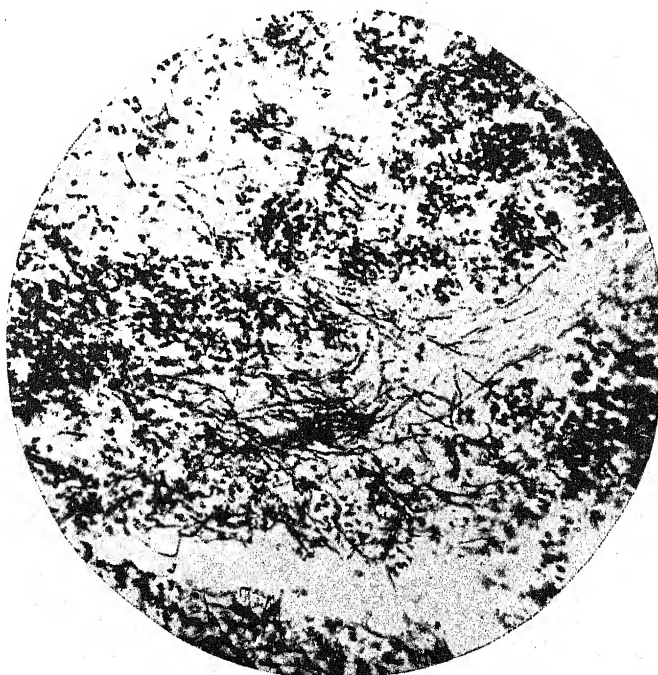
FIG. 7. Section of intestines showing long mycelial forms and numerous yeast forms. (Gram's stain) ×500.

course of their passage through the bowels were evidently taken up by the pha-
gocytes in the injured part. Thus once established, the lesions increased giving
rise to inflammation, abscess formation and the subsequent chain of events
already described. From the intestinal lesions, the infection spread to the mesen-
teric glands by way of the lymphatics. The punctured foot observed a few
months before the animal was taken ill had apparently no relation with the causa-
tion of this condition, for the reason that the parasites, even if they had been
taken up through the puncture, could not have infected the bowels and mesen-
teric glands primarily to the exclusion of the cutis and its lymphatics.

That Runciman and Harber observed cutaneous lesions appearing all of a
sudden in enormous numbers in horses which had been ailing for some time has
been mentioned, and the primary seats of infection in such cases are no doubt
in the internal organs. In such cases, it is possible that the organisms gain entry
into the blood vessels, as often happens in amoebiasis, and are carried as emboli
and deposited in all parts of the body. Their spread by way of the lymphatics
could not have produced such an enormous crop of lesions all of a sudden to the
exclusion of the numerous lymphatic glands, which act as filters and arrest the
progress of the organisms. Hence spread by way of the blood stream appears
to be the only probable method but this requires confirmation.

SUMMARY

An account is given of an atypical form of epizootic lymphangitis affecting
the intestines and mesenteric glands of a mule which died showing symptoms
of enteritis. The condition was not diagnosed during the life due to the absence
of any cutaneous manifestation and the animal was treated for worms only.
A short description is given of the clinical symptoms noticed during life and the
lesions encountered on *post mortem* examination. A brief review is given of the
relevant literature including that dealing with atypical forms of the disease.
Histopathology of the lesions and the microscopical features of the parasites
are described. The methods of infection and spread of the lesions in this disease
are also discussed.

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SOME NOTES ON CUTANEOUS MYIASIS IN ANIMALS IN THE MADRAS PRESIDENCY

BY

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Cutaneous myiasis, though common in the domesticated animals in India, has not, it would seem, attracted much attention from veterinary workers in this country. Some medical workers have recorded such conditions in man, but Patton [1921, 1922] appears to be the only one among them, who has published some notes on the Indian Calliphorinae which cause cutaneous myiasis in man and animals. He records that *Chrysomia bezziana* Vill. is the commonest cause of such conditions. Besides this fly, which is an obligatory myiasis producer, he found larvae of *Chrysomia megacephala* Fabr. in the sores of three bovines, and those of *Lucilia argyricephala* Macq., in three other bovines in the Bombay Presidency. These two flies usually breed in dead meat, but may cause cutaneous myiasis though perhaps rarely.

The writers obtained maggots both alive and preserved, from fly-blown sores of domesticated animals in this Presidency for one year, commencing from December 1934 to the end of November 1935. The live larvae were despatched from the districts in dry earth, and by the time they arrived in the Laboratory by post, they had pupated. The puparia were kept in suitable receptacles and the flies that hatched out were collected for study. In all, 404 specimens were received from different animals, each sample containing 10 to 15 larvae. The study of these showed that 401 specimens were of *Chrysomia bezziana* and the other three of *Lucilia argyricephala*. The last three were obtained from bullocks, in two cases from sores on the neck and at the base of a loose horn of the third. These findings confirm the work of Patton, except in regard to myiasis caused by *Chrysomia megacephala.* Some breeding experiments with *Chrysomia bezziana, C. megacephala* and *Lucilia argyricephala* were done in this Laboratory and it was found that *Chrysomia bezziana* is purely obligatory in wounds, whereas the other two bred easily in dead meat.

The present work has made it possible to obtain some knowledge regarding the seasonal prevalence of these flies and the areas in which they abound in this Presidency. This knowledge may be useful in adopting measures to lessen the incidence of cutaneous myiasis in animals or man in those areas, at certain seasons.

It is interesting to note that maggots of *Chrysomia bezziana* were received from sores on four fowls and two sheep. The maggots from the fowls were collected from the sores on the cloaca in all cases and came from four different localities more or less widely separated from one another, *viz.*, Dindigul, Cannanore, Anantapur, and Hospet. The first three were obtained in December and January and the last one in June. The maggots from sheep came from Anakapalle and Jeypore, both in Vizagapatam District. They were collected from a sore on the ear of one and from that on the neck of the other. It would appear that cutaneous myiasis caused by *Chrysomia bezziana* in fowls and sheep has not been noticed in this Presidency, possibly in India, and this opportunity is taken to place the fact on record.

TABLE I

Regions of the Sores from which maggots were collected	Camels	Bovines	Buffaloes	Goats	Sheep	Equidae	Dogs	Fowls	Total
Base of hoof	...	31	3	1	...	2	...	...	37
Base of horn	...	14	7	2	...	...	...	...	23
Mucous surfaces	1	78	33	7	1	6	2	4	126
Skin	...	132	42	9	1	14	19	...	218
Total	1	250	85	19	2	22	21	4	404

N.B.—In this table are included the three bovines from which larvae of *Lucilia argyricephala* were collected.

Table I shows the species of animals and the situation of sores from which maggots were collected. The number of bovines and buffaloes infested seems to be large owing to the fact that the majority of the Veterinary patients are drawn from these classes of animals. It would appear that sores on the skin and on the mucous surface of the genitals, etc., are more prone to be fly-blown than those in other situations in any class of animal.

TABLE II

Rainfall averages			Number of specimens	Names of Districts.
Agency Plains .	.	59·0 45·1 }	38	Ganjam
Agency Plains .	.	59·5 40·2 }	14	Vizagapatam
Agency Plains .	.	49·0 40·3 }	26	Godavari East
		39·3	*Nil*	Godavari West
		37·0	10	Kistna
		32·1	1	Guntur
		35·0	*Nil*	Nellore
		28·2	23	Cuddappah
		25·7	13	Kurnool
		23·2	7	Bellary
		22·7	4	Anantapur
		33·5	17	Chittoor
		46·5	10	Chingleput
		46·0	4	South Arcot
		37·7	18	North Arcot
		32·7	16	Salem
		26·6	56	Coimbatore
		74·1	*Nil*	The Nilgiris
		117·9	37	Malabar
		145·5	57	South Kanara
		44·9	14	Tanjore
		33·8	10	Trichinopoly
		31·4	11	Madura
		30·7	18	Ramnad.
		29·0	*Nil*	Tinnevelly

Table II shows the average rainfall and the number of specimens received from each district. None was received from Godavari West, Nellore, Nilgiris and Tinnevelly districts. The fluctuation in the number of specimens from each district may be due to lack of interest in those concerned in collecting specimens. Hence it is difficult to say which of the districts has a larger incidence, but as one would expect, a large proportion of the specimens was received from South Kanara and Malabar on the West Coast where rainfall and humidity is great, and a small number from dry districts like Anantapur.

H

TABLE III

Names of months	Number of days taken by the puparia to hatch
December 1934	8
January 1935	8
February 1935	8
March 1935	7
April 1935	7
May 1935	None hatched.
June 1935	None hatched.
July 1935	7
August 1935	7
September 1935	8
October 1935	8
November 1935	8

Table III shows the average number of days taken by the puparia to hatch out flies during the respective months of the year. It is seen that from September to February, which is the cooler half of the year in Madras, the average time taken by the puparia to hatch is 8 days. In the months of March, April, July and August the time taken was seven days whereas, in the months of May and June which are the hottest months, none hatched. It was observed that the number of flies that hatched out from the puparia decreased gradually from February to April and began to increase from July. During the months of December and January, over 95 per cent of the puparia were productive.

TABLE IV

Names of months	Number of specimens received
December 1934	105
January 1935	84
February 1935	57
March 1935	80
April 1935	19
May 1935	9
June 1935	18
July 1935	14
August 1935	8
September 1935	7
October 1935	16
November 1935	7

Table IV shows the number of specimens received each month, commencing from December 1934 to the end of November 1935. Here again, there are factors which prevent one from arriving at any definite conclusion regarding seasonal incidence of the flies. Hence, with a certain amount of reserve, one would venture to say that the large number of specimens received from December to the end of March probably indicates this as the optimum season for *C. bezziana*. The fall in the number of specimens during the hot season may be attributed to less flies hatching out of the puparia. Perhaps this is a natural control against over-population of *C. bezziana*. It would seem, therefore, that cutaneous myiasis in animals in this Presidency prevails mostly during the cool season of the year. The present work makes us believe that no "sheep maggot fly" (*Lucilia sericata*) exists in this Presidency. The maggots received from the two sheep referred to were of *Chrysomia bezziana*.

There is another class of flies (Oestridae) which produces myiasis in the nasal chambers, stomach and intestines of animals, these are *Oestrus ovis*, *Gastrophilus equi* and *Cobboldia elephantis*, the larvae of which are received here for identification, from time to time, from sheep, horses and elephants respectively in this Presidency. So far no larvae of *Hypoderma bovis* or of *H. lineata* have been recorded from indigenous bovines of this province. There are two instances of larvae of *Hypoderma* species being removed from a Tibetan yak and from some imported horses at Ootacamund, and sent here for identification. These animals evidently were infested with the larvae before arrival in South India. *Oestrus ovis* on the other hand seems to be fairly common here.

It is interesting to record that, recently, a sample of fresh faeces containing a large number of maggots was brought to this Laboratory with the history that they were passed by a thorough-bred race horse. The maggots pupated within 24 hours after their arrival and a large number of *Musca nebulo* were hatched out of the puparia. If the history is correct, it would appear that these maggots which may have been accidentally ingested by the horse, passed through the intestinal canal without any damage to themselves. A few years ago a sample of faeces containing maggots, passed by a hound, was received from Khallikote. The hound had severe diarrhoea at the time. The maggots were received dead, and they resembled those of *Musca nebulo*. It would seem, therefore, that the larvae of *Musca nebulo* can cause accidental intestinal myiasis in animals.

SUMMARY

1. The most common cause of cutaneous myiasis in animals including fowls and sheep is *Chrysomia bezziana*. On three occasions only maggots of *Lucilia argyricephala* were obtained from sores on bovines.

2. "Sheep maggot" fly (*Lucilia sericata*) and Hypoderma species have not been recorded in this province.

3. The possibility of the larvae of *Musca nebulo* causing accidental intestinal myiasis is recorded.

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SECRETION AND COMPOSITION OF PAROTID SALIVA IN BUFFALOES

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Most of the work on secretion and composition of saliva has been done on the dog, although some work has been done on the horse, ox, sheep, goat and pig ; but controversy still exists on certain points. No attempt, however, appears to have been made on the buffalo. It will, therefore, be not without interest to record some experiments made on this animal.

PROCEDURE

(*a*) All experiments were conducted on a buffalo-cow with a salivary fistula of the left parotid duct.

(*b*) The animal was, in each case, secured in stocks and the head kept in a fixed position to ensure uniform flow of saliva.

(*c*) All injections were made subcutaneously.

(*d*) The quantity of saliva mentioned in the text was collected in five minutes' time for all experiments.

(*e*) Saliva for the examination of the physical character and composition was collected before the injection of drugs from the parotid gland in a resting state.

EFFECT OF PHYSIOLOGICAL REFLEXES

It is not essential that the food should enter the mouth to cause the flow of saliva. The sight or odour or even the thought of food, provided the individual be hungry, calls forth salivary secretion ; the mouth is said to water. Pavlov [1910] has extensively studied the psychic salivary secretion in the dog, and has shown that the sight of meat calls forth a stringy saliva from the sub-maxillary and sublingual but none from the parotid, whereas the sight of dry meat powder or bread calls forth an abundant secretion of parotid saliva. Colin [1871] states that in herbivora the secretion of saliva is uninfluenced by the sight or smell of the foods. Scheunert and Trautmann [1921] remark that psychic secretion is slight in sheep, and it is probable that it is small or absent in all herbivora.

It appears from the experiments made on the buffalo that slight psychic reflex exists, but it is not as much developed as in the dog. The sight of food slightly increased the outflow of parotid saliva from 30 mls. to 36 mls.

The animal with salivary fistula of the parotid duct was fed on green grass. The quantity of saliva collected during mastication was 42 mls., but when animal was fed on dry *bhoosa* there was a copious flow of saliva measuring 102 mls. The character of the food appears to play an important rôle on the parotid secretion, and dry food excites production of a larger quantity of saliva.

ACTION OF DRUGS

Two grains of pilocarpine nitrate and a grain and a half of arecoline hydrobromide were injected at an interval of 24 hours between each. They produced a copious flow of saliva five minutes after injection. Atropine is an antagonistic to pilocarpine, and when injected in one-grain dose it markedly reduced the secretion. The action of the drugs on the parotid secretion is shown in the following table :—

TABLE I

Drug	Quantity of saliva collected for a period of 5 minutes				
	Control	5 mins. after the injection	15 mins. after the injection	25 mins. after the injection	35 mins. after the injection
	Mls.	Mls.	Mls.	Mls.	Mls.
Pilocarpine nitrate	35	132	135	135	134
Arecoline hydrobromide	32	115	112	114	110
Atropine sulphate	30	14	12	10	10

Pilocarpine stimulates the parasympathetic nerve endings and thus an excessive secretion is produced. Atropine on the other hand paralyses the terminations of the secretory fibres of the chorda tympani and reduces or inhibits secretion. This is not due to any action on the salivary cells, but to the failure of nerve impulses. Pilocarpine injected after a large dose of atropine failed to produce excessive secretion.

PHYSICAL CHARACTER AND COMPOSITION

The saliva was clear, colourless, watery and translucent, having a specific gravity of 1008. The reaction was alkaline and the pH. was 8·8, which was determined by the use of the colorimetric method.

The saliva contained no mucin, but a small quantity of protein was present.

TABLE II

Thousand parts by weight of parotid saliva contain

Saliva	Water	Total solids	Organic matter	Inorganic matter
Parotid saliva . . .	991·5	8·5	1·7	6·8

The results of chemical analysis of the inorganic constituents are given in the following table.

TABLE III

Thousand parts by weight of saliva contain

Saliva	Sodium	Magnesium	Potassium	Sodium, as chlorides
Parotid gland . . .	2·768	0·06	In traces .	0·098

Total chlorides, 0·154 ; sulphates 0·145 ; and phosphates, 3·590 were present in 1,000 parts of saliva by weight.

Ptyalin is an amylolytic enzyme present in the saliva of man and in some domesticated animals ; it is capable of converting starch into dextrin and maltose.

Ellenberger states that both the parotid and sub-maxillary secretions of horse and ox can convert starch into sugar. Smith [1890] says that in all domestic animals the parotid saliva possesses the highest degree of amylolytic power. In ruminants the diastatic action of saliva is about the same as that of the horses. Schwarz and Steinmetzer [1924] say that the saliva of ox contains no amylase. Palmer [1916] states that the amount of ptyalin present in the saliva of the ox is insignificant.

From the review of the literature it is concluded that there is a difference of opinion about the presence of ptyalin in the saliva of ruminants.

The presence of ptyalin in the parotid saliva of the buffalo was studied by Evans' method, and it was observed that the starch was neither converted into erythrodextrin nor into maltose when subjected to the action of the parotid saliva from the buffalo.

Further experiments were conducted to determine whether the parotid saliva of the buffalo contained a very small quantity of ptyalin, which may require a longer time to exert its action on the starch. Both boiled and unboiled solutions of starch were mixed with an equal quantity of saliva in two separate tubes and allowed to remain at 38° C. for two hours. At the end of this period neither erythrodextrin nor maltose could be detected in either of the tubes. The solutions were allowed to remain at room temperature for 24 hours in presence of toluene but the starch remained unchanged. The experiments were repeated several times with identical results. It can reasonably be concluded from these experiments that ptyalin is absent in the parotid saliva of the buffalo.

ACKNOWLEDGMENT

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SELECTED ARTICLE

THE INHERITANCE OF PRODUCTIVITY IN FARM LIVE STOCK*

1. MEAT

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Introduction.—Almost all the characters that are of any importance for meat (such as weight-for-age and body-proportions) in our farm animals are dependent for their full expression on environment and nutrition. We cannot, therefore, consider the genetic characters for meat-production without considering the environment in which they are developed. In my opinion, most of these characters have been developed purposely, and their development has been planned and directed by man through selection in an environment that he has created to produce them, whilst in only a very small minority of cases have they arisen, by chance, from large mutations. The large mutations that occur in our live stock are nearly all of the recessive type, and for the most part consist of defects and abnormalities or fancy points (such as colour and horns) which are of little commercial importance. They usually segregate out in simple ratios, and it is an easy matter to breed for them by using Mendelian methods. On the other hand, almost all the commercial qualities are ' blending ' in inheritance : there is no dominance, and in my opinion they have been produced by quite a different method, that is, by the accumulation of small variations, which are contiunally appearing, and may be stimulated by the environment. In my opinion, too, these characters exist in all degrees of ' fixity ' in the animal. In other words, in terms of present-day explanations, I believe not only in the mutation of a gene already formed, but also in the possibility of the evolution of a new gene by the animal itself under the stimulation of the environment, and consequently of varying degrees of ' fixity ' of characters under environmental change, according to the state of evolution of the gene in question. Whilst the animal produces the gene as a mechanism for putting the characters that it has evolved into its inheritance, a mutation in the gene already fully formed gives a variation in the animal that is of small importance in evolution, because it is at random and not purposeful, as in the former case. Thus, I see the real evolution of commercial

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* The five papers under this head were read to Section D (Zoology) of the British Association for the Advancement of Science, Aberdeen, September 10, 1934.

qualities, built up by small variations, constantly being added to according to the environment of the animal, and the formation of varieties, freaks, and fancy points produced by the mutation of genes already formed by the other process.

To illustrate the theories outlined above, I propose, in the short space available, to give a few examples.   A concrete example may be given from the horse, which although not among the meat-producing animals in Great Britain is closely related to them.   The evolution in the skeleton (Fig. 1, Plate XIV) has followed a definite and uniform course of changes, consisting in the main of a progressive lengthening of the limb bones in relation to cranium size.   This evolution has not been broken by a number of sharp changes, such as mutations affecting different parts of the body independently ; for   example, no shortened limb bones, such as those which occur in the Dachshund dog (and also occasionally in the horse) and behave as Mendelian recessives, come in   the series.   These things are mutations that may easily be picked out by man and bred to form fancy strains, but they play no real part either in natural evolution or in the development of the proportions of the body in commercial meat-production.   These mutations do not, as a rule, form intermediates when bred to the normal type, whereas when two different ' developed ' types are crossed, all gradations between them may be obtained.   In the horse (see Plate XIV) the different types of conformation are magnifications (light horses) or extensions (heavy horses) of the gradual changes which have taken place during the course of evolution.

Cattle (beef and veal).—Beef qualities, *i.e.*, a high proportion of the best joints (loin) and a low proportion of the offal parts (head and legs), are developmental characters, and change in their proportions as the animal grows up (Fig. 2, Plate XV).   The head and legs are proportionally large in the calf, and as the beef qualities develop they become proportionally smaller, and the loin becomes proportionally larger.   For the full expression of these developmental characters, a high plane of nutrition is necessary, for, if it is not available, the later maturing and more valuable parts are not developed and the form of the animal approaches that of the unimproved type in which the head and legs are large and the loin poorly developed (see Plate XV).   If selection is made under poor conditions of nutrition, therefore, we cannot distinguish so well between the one which is poor in conformation due to lack of genetic improvement, and the one which is genetically improved but fails to develop its body proportions because of lack of nutrition. All the best breeds of beef cattle (Aberdeen-Angus, Shorthorn, Hereford) have been developed in areas of good nutrition, and herds in poor nutrition (range) areas become degenerate from a meat point of view, unless they are kept constantly supplied with breeding-stock which has been selected in the areas of high nutrition.

When we consider the different breeds of cattle from the standpoint of the development of body proportions for meat (Fig. 3, Plate XVI), it will be seen that

they can be put in a series according to the rate and extent to which these proportions are developed. A beef Shorthorn bull 14 months old is as well developed in its proportions for meat as an adult Friesian bull $5\frac{1}{2}$ years old, and the extent to which it develops eventually is far in excess of anything found in the dairy breeds. The directive influence of man's selection is seen in the way in which stocks having a different origin—such as the Aberdeen-Angus and the Shorthorn—approach one another in conformation for beef purposes (and Friesians and Dairy Shorthorns for milk purposes), whilst stocks with a common origin—such as the Shorthorn—have developed different types when bred for different purposes, such as beef and milk (see Plate XVI).

Of quite another nature genetically, and in no way dependent on environmental and nutritional conditions, is the 'Doppellender' calf, which is so much valued for veal on the Continent (Fig. 4, Pl. XVII). It consists of a doubling of the muscles of the loin and hind quarters, and has arisen as a mutation in several Continental breeds of cattle, in which it is carried on in the heterozygous condition, for the females are sterile. It is a simple recessive and forms no blend in crossing as do the developmental characters. It cannot, therefore, be used to improve other stocks in the same way that Aberdeen-Angus bulls are used to introduce an improved conformation and better quality when mated to coarse-boned and ill-proportioned cows.

The colour of the body-fat in cattle is a multiple-factor genetic character; all shades of colour exist from a very pale yellow (which is desired by the butcher) to a deep yellow (which is desired by the breeder of dairy cattle). The expression of this character is dependent on the amount of the xanthophyll pigments of plants in the food, and if these are absent the fat becomes white, no matter what the genetic constitution is. In selecting for this character the breeders of dairy cattle feed plenty of greenstuff and pick out those cows which give the deepest yellow tint. Variability curves (by Whetham) for the colour of the butter-fat in the different breeds of cattle at the London Dairy Show are shown in the next figure (Fig. 5, p. 274). This is a case where a definite environment of food-supply is necessary before selection can be made for the genetic character concerned.

Sheep (mutton and lamb).—As with beef, the genetic characters for mutton and lamb are all 'developmental' ones, and in Great Britain there exists a complete range of types from those possessing early maturing qualities (that is, a quick change in the proportions of the body), and suitable for killing as lamb, to those with a slow change in proportions, which are more suited to mutton-production. These characters are not firmly fixed, however, and may be modified in any one breed by the methods of feeding and management adopted. During growth, the tissues of the body develop in a definite order—bone, muscle, and then fat. The proportional development of these tissues varies considerably in different breeds;

this, for example, may be illustrated by the following average fat-measurements of the loins (Fig. 6, Plate XVII) of carcasses at Smithfield Show (Fig. 7—by Hirzel, p. 275). First crosses are intermediate between parents in this respect, and there is no dominance or recessiveness of such developmental characters.

On the other hand, an alteration of the proportions of the body brought about by a mutation (and not of a developmental character), such as that of the short legs of the Ancon sheep (Fig. 8, Plate XVIII), behaves as a recessive segregating character, and does not blend with all gradations as do the developmental characters. The developmental characters must always be represented by a variability curve, and never as a fixed point, as the mutations can be. Improvement of the environment and selection of the animals at the upper end of the curve, for the curve shifts upwards as the result of environmental changes, are the best means of effecting improvements for early maturity.

Pigs (bacon, pork and lard).—Local feed conditions have supplied the environment in which the different types of pigs have been evolved. For example, in the maize-producing areas of Hungary and Roumania, the Mangalicza pig has been developed for fat-production; this pig has only a small development of bone and muscle, but the back fat, which it is bred for, reaches, in a good average pig of 390 lb. live-weight, a thickness of 5 in. at the shoulder and 4 in. at the loin. In the corn-belt of America, too, the Poland-China breed developed in the same way (Fig. 9, Plate XVIII) but the type within the breed has been changed in recent years (owing to the lack of demand for lard) by selection, and the much greater use of proteins in the ration. Under the feeding conditions existing in Denmark (skim milk and cereals), the bacon pig (in which a carcass of 150 lb., thick in lean, and with only a moderate amount of fat, is required) has reached its highest development. Progeny test of growth and carcass quality, made under feeding conditions that stimulate the characters required, are the means whereby this has been achieved. It has been a directed evolution of commercial qualities by the accumulation of small increases (as the progeny tests of their boars show), and not by the appearance of sudden large mutations. For pork (where a large development of the muscle at a low carcass weight—70 lbs.— is required), it is in environments supplying a ration high in protein that the highest perfection is attained. New Zealand, for example, with its large supply of skim milk and meat meal is producing this type of pig to perfection from breeds as diverse as the Berkshire, Large White, and Tamworth (see Fig. 10, Plate XIX). The high-protein and low-carbohydrate feed leads to the development of the muscle and limits the fat to the proportions required by the consumer, whereas under our conditions of high-carbohydrate feeding the differences in fatness between these breeds would be very marked. In general, the degree of fixity of the commercial character will depend on the order of its development in the animal; thus bone, which develops first, is more difficult to modify the local feeding condition than is fat, which
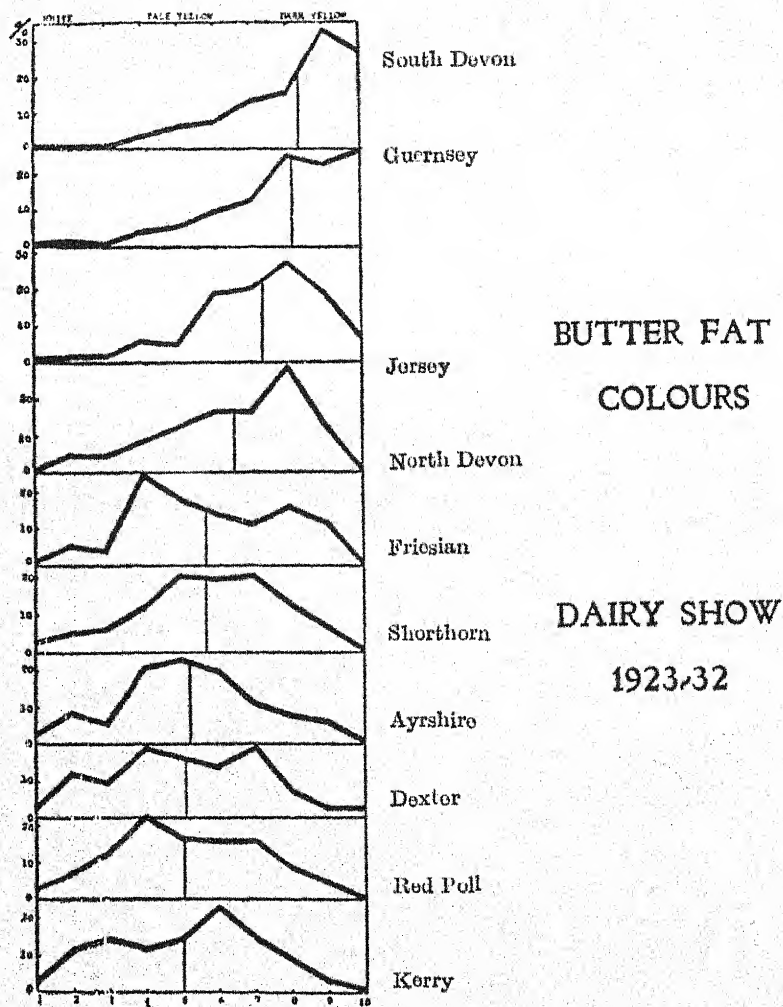
Fig. 5. (From Whetham—paper in preparation.)

Variability Curves of the Colour of Butter-fat in different Breeds of Cattle.—London Dairy Show data (1923-32). Range of colour measured on a butter-fat colour scale. 1 = white, 10 = dark yellow. The vertical lines show the mean values for the different breeds.

THICKNESS OF FAT OVER LOIN (C) 9 MONTHS
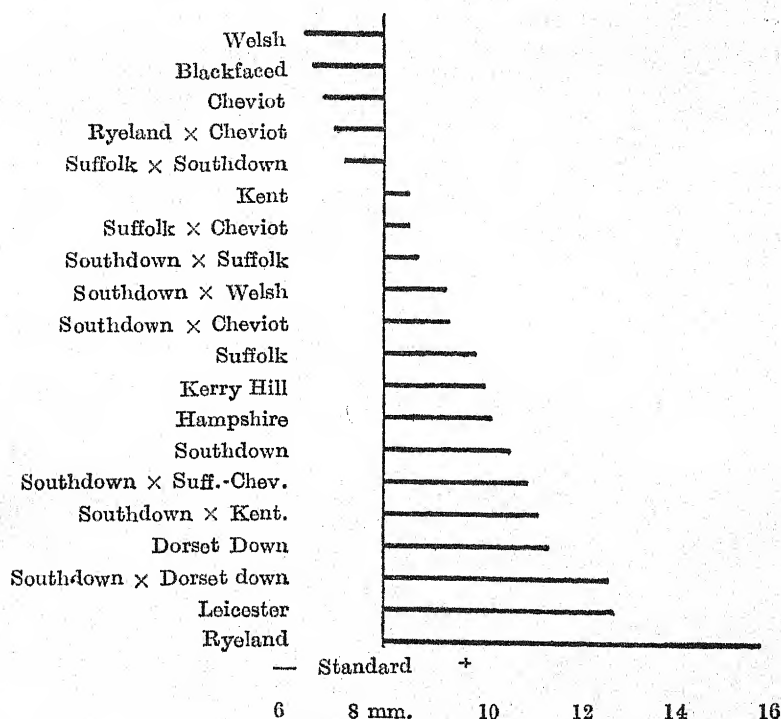
Fig. 7.   (From Hirzel—paper in preparation.)

Measurements of Fat over the loin in different Breeds and Crosses of Sheep at Smith-field Show.—For position of measurement see Fig. 6, Plate XVII.   The measurements are shown as — or + the optimum measurement (8 mm.) desired by the public, and are breed averages over a number of years.   Approximate age of carcasses—9 months.

develops last.   The developmental meat characters of the animal are genetically fixed only in relation to a certain environment.   Conversely, if we wish to develop a certain character in our stock, we must carry our selection for that character in a suitable environment, if we are to be successful.

General conclusions.—Since the genetic characters concerned in meat-production are so dependent for their expression on the environment, especially nutrition (and are mostly of a developmental character), our best means of directive improvement is selection (by progeny tests) in a suitable environment, that is, one which

stimulates the development of the character in question. The further development of these commercial qualities in our animals depends, like the ' civilization qualities ' in man, on the creation of a better environment for the development of the characters concerned.
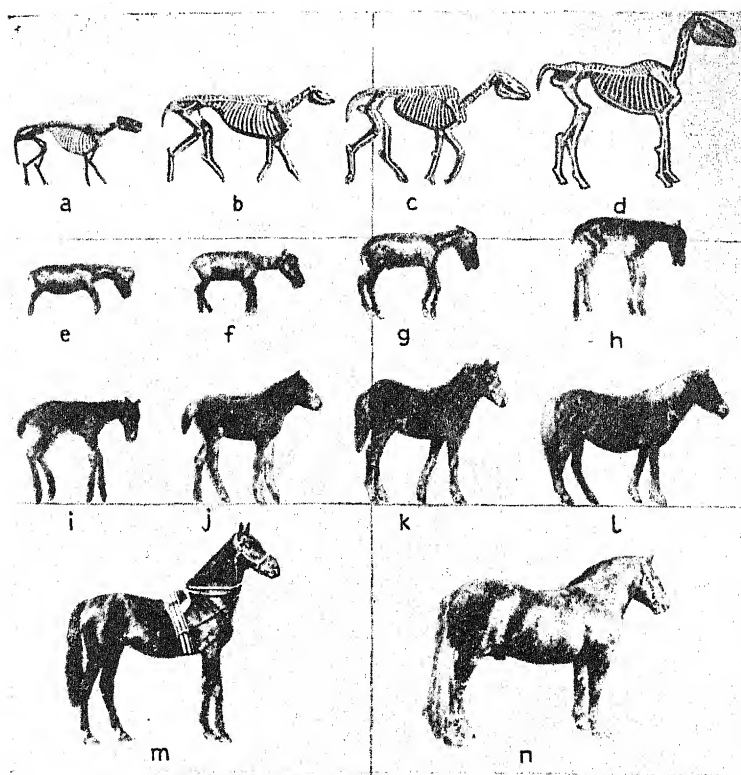
FIG. 1. THE CHANGES IN THE PROPORTIONS OF THE
HORSE IN DEVELOPMENT AND DURING
EVOLUTION

In order to show the changes in proportions, all the photographs have been reproduced to the same cranium size (eye to ear length). The changes in proportions during embryonic life parallel those which have taken place during evolution.

Reading from left to right :

Top line : EVOLUTION—(*a*) Eohippus ; (*b*) Mesohippus ; (*c*) Merychippus ; (*d*) Equus (Arab).

Second line : DEVELOPMENT (Welsh Pony)—(*e*) 3 months ; (*f*) 5 months ; (*g*) 7 months ; (*h*) 10 months.

Third line : DEVELOPMENT (Welsh Pony)—(*i*) 11 months ; (*j*) 2 weeks after birth ; (*k*) 9 weeks after birth ; (*l*) adult.

Bottom line : EVOLUTION—(*m*) Light Horse (Thoroughbred—St. Simon) ; (*n*) Heavy Horse (Suffolk—Wedgwood).

*(From Hammond, 'Actes, 16ᵉ Cong. Internat. d'
Agric., Budapest,' 1934.)*
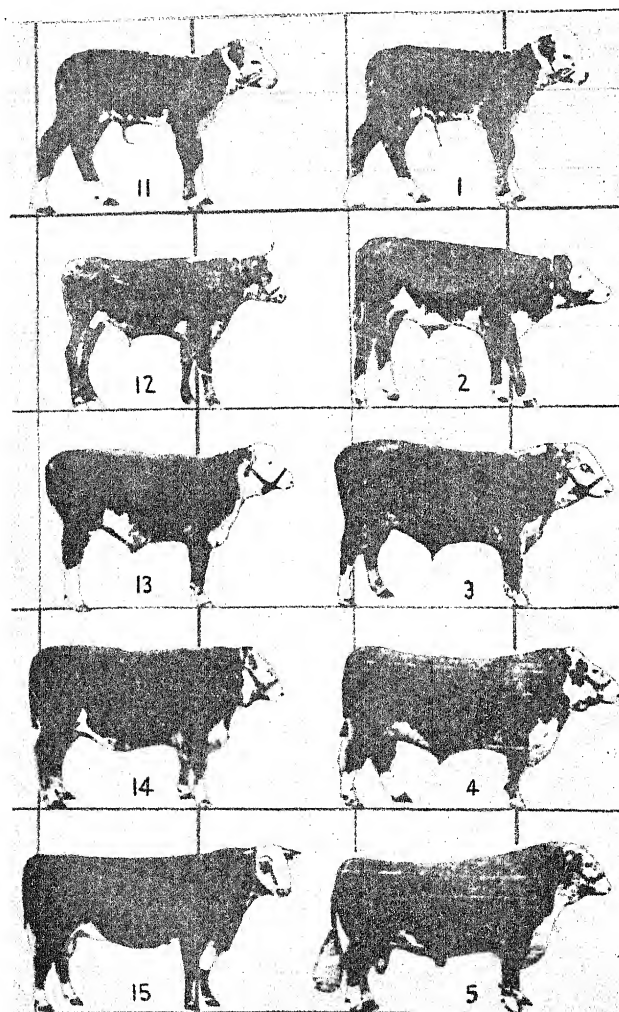
FIG. 2. CHANGES IN THE PROPORTIONS OF THE BODY IN
HEREFORD CATTLE

In order to show changes in body proportions, as distinct
from size, the photographs have all been reduced to the same height
at the shoulders, so that the proportions are shown in relation to
this measurement.

11. Heifer—2 days old.

12. Steer—30 months old (reared
on low plane of nutrition).

13. Steer—11 months old (reared
on high plane of nutrition).

14. Steer—22 months old (reared
on high plane of nutrition).

15. Bull—adult—type existing
100 years ago.

1. Heifer—2 days old.

2. Bull—5 weeks old.

3. Bull—13 months old.

4. Bull—22 months old.

5. Bull—5 years old.

(*From Hammond, 'Actes, 14ᵉ Cong. Internat. d' Agric., Bukarest,' 1929*)

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PLATE XVI.
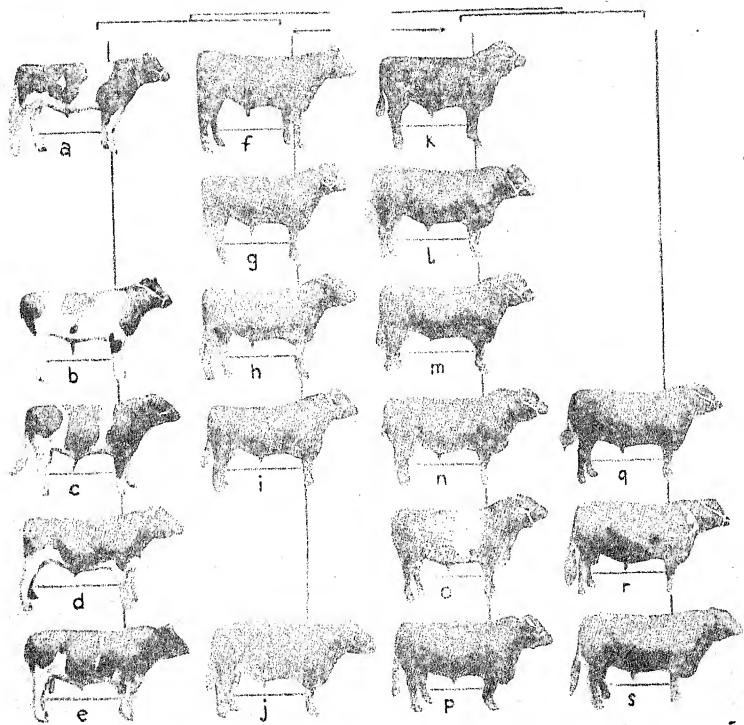
FIG. 3. PROPORTIONS AND AGE CHANGES IN BULLS OF
DIFFERENT BREEDS

1st Prize Bulls at the Royal Agricultural Show, Manchester, 1930 ;

*Convergent
Evolution*

*Divergent
←Evolution→* Beef

Beef

Milk

Milk

Beef

Beef

Friesian

Dairy Shorthorn

Beef Shorthorn

Aberdeen Angus

(a) 12 months	(f)	12 months	(k)	10 months	—
—	(g)	13 ,,	(l)	14 ,,	—
(b) 18 months	(h)	16 ,,	(m)	16 ,,	—
(c) 21 ,,	(i)	22 ,,	(n)	26 ,,	(q) 19 months
(d) 25 ,,	—		(o)	28 ,,	(r) 31 ,,
(e) 5½ years	(j)	4½ years	(p)	5½ years	(s) 5½ years

(Photographs all reduced to the same shoulder-height.)
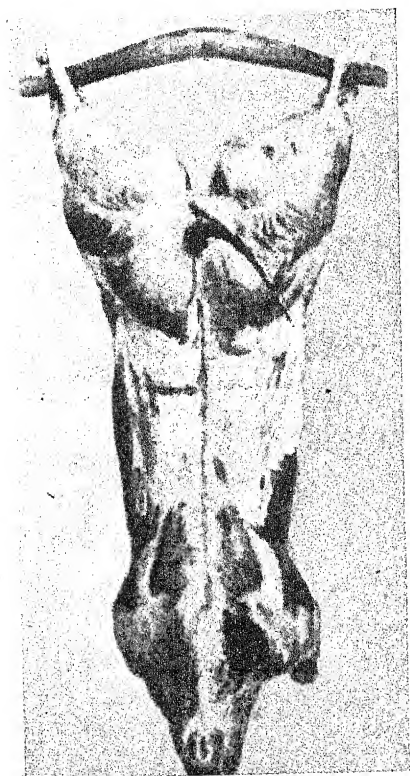
FIG. 4.

CARCASS OF A 'DOPPELLENDER'
VEAL CALF

11 weeks old. Weight 367 lb.

(*From Herter and Wilsdorf, 'Arbeit deut.
Landw.-Gesell.' ; H. 206, Berlin, 1912*).
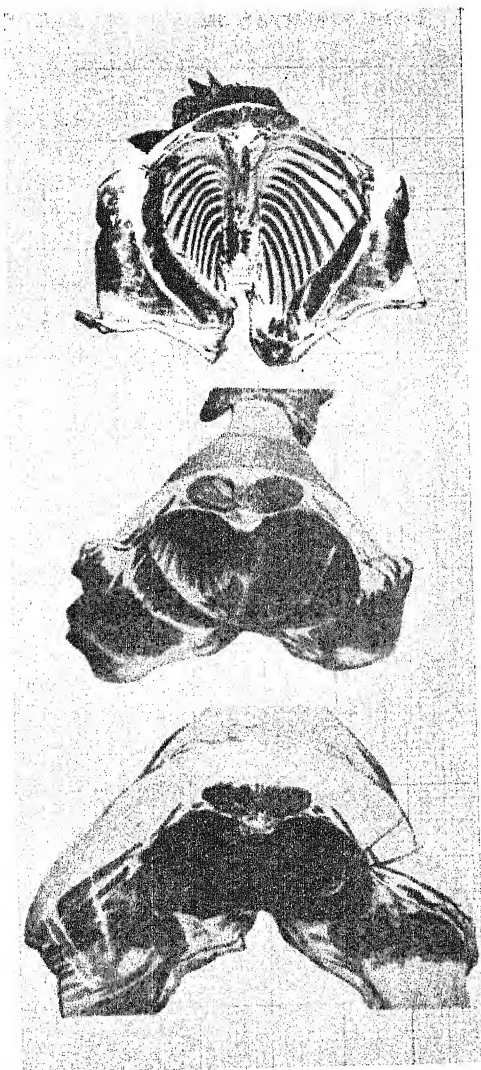
FIG. 6. LOINS OF MUTTON (cut at last rib)

To show where the fat-measurements given
in Fig. 7 were taken—at the narrowest part
over the 'eye' muscle. The three carcasses
illustrate :

Top : A carcass with too little fat and an
insufficient thickness of 'eye' muscle.

Middle : A carcass which has the propor-
tions of fat and muscle required by the
public.

Bottom : A carcass in which the fat is much
too thick.
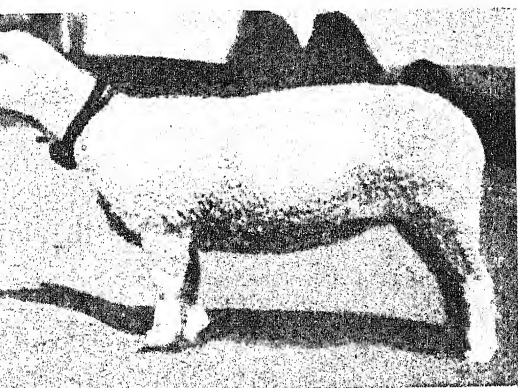
FIG. 8.
THE ANCON SHEEP

The shortened legs are a recessive mutation and do not give intermediates on crossing with the long-legged type, as occurs when a breed with 'developed' short legs (such as the Southdown) is crossed with a long-legged type.

(*From Wriedt, 'Heredity in Live Stock', London, 1930.*)
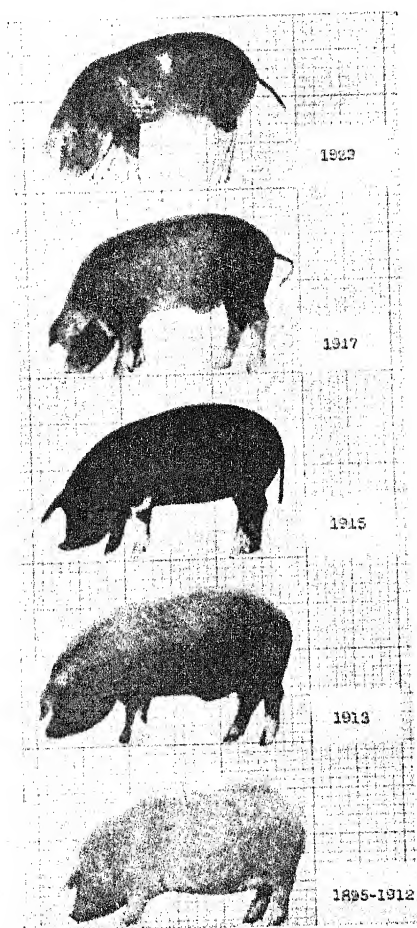
FIG. 9. CHANGES MADE IN THE POLAND-CHINA PIG WHEN THE DEMAND FOR LARD DECREASED

All the photographs have been reduced to the same shoulder-height to show changes in proportions as distinct from size. The breed has been changed by selection in the direction of later maturity, *i.e.*, with more bone-and muscle-growth and less fat. These successive changes are the reverse of the changes which occur as the pig grows up. This has been attended with increase in actual size.

(*From Hammond, F. Roy. Agri. Soc., 1932, 93.*)
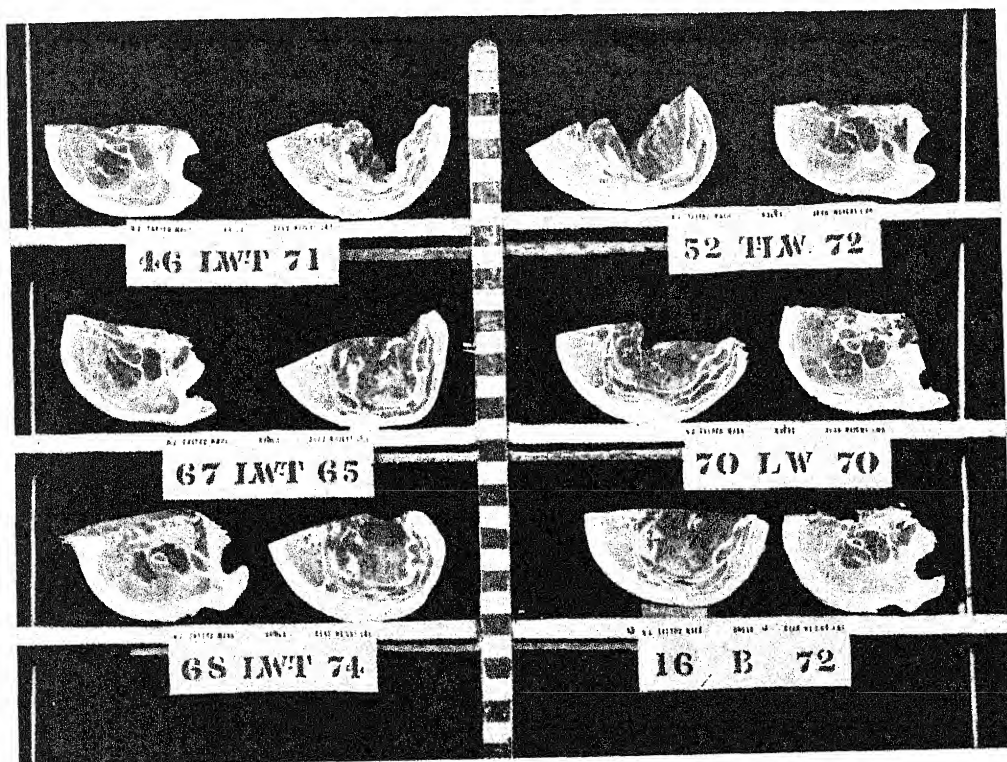
Fɪɢ. 10. CUTS THROUGH THE LEGS AND LOINS OF NEW ZEALAND
PORKERS OF DIFFERENT BREEDS AND CROSSES

Nos. 46, 67, and 68 : Large White × Tamworth ; carcass weight, 71, 65, 74 lb.

No. 52 ; Tamworth × Large White ; carcass weight, 72 lb.

No. 70 : Large White ; carcass weight, 70 lb.

No. 16 ; Berkshire ; carcass weight, 72 lb.

Note on the loins the thickness of the 'eye' muscle, the uniformity and
thinness of the fat over the 'eye' muscle, and the thickness and well-
developed lean meat of the 'streak'.

(From photographs taken for the Department of Sci. and Ind. Research, Wellington,
New Zealand.)

THE INHERITANCE OF PRODUCTIVITY IN FARM LIVE STOCK

II.—MILK

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(Institute of Animal Genetics, University of Edinburgh).

Can the science of genetics offer reasonable help to the live-stock producer ? If so, then by what means ? In this paper it falls to me to deal with these two questions from the point of view of milk-production in cattle.

Before the scientist can tackle any problems he must be able to weigh or to measure that with which he is dealing. Both quantity and quality must be assessed. Thus genetical experiment in respect of eggs and milk is much easier than it is in the case of meat.

As Dr. Hammond has so clearly stated, it is essential to be able to discriminate between the factors affecting the productivity of an animal that are due to environment, nutrition, management, disease, etc., from those factors that are genetic. The difficulty of doing so is one of the rocks upon which the barque of the geneticist is most likely to founder ; and the task is further complicated because attempts to correct for environmental and other variations are likely to mask a genetic factor. For instance, Mackintosh [1] has recently shown that, where it is necessary, for comparison, to correct the first-lactation-yield of dairy cattle, the correction factors employed often work reasonably well, but that in the case of one group of cows they were very far out.

Up till now, about 400 papers have been written dealing principally with some aspect of the inheritance of milk-yield. Some useful information has been obtained, but that source is now becoming exhausted, and I think that any future information of scientific value regarding the inheritance of yield and quality of milk will be obtained only by deliberate experimentation. By deliberate experimentation I mean the control of environment and nutrition to the greatest possible extent, so that, although the production of one generation takes place many years after the production of that ancestral generation with which it is to be compared, the comparison may be as straightforward as possible and with the minimum use of correction factors.

The need for this has been recognized by Dr. Graves and those in charge of the experiments conducted by the Bureau of Dairy Industry of the United States Department of Agriculture, and Dr. Lush informs me that several of the State agricultural experiment stations, such as Illinois and Nebraska, have now also laid down similar experiments with dairy cattle. This principle forms the basis

of the experimental work that we are conducting with dairy cattle and pigs at the experimental farms of the Institute of Animal Genetics. The method adopted is to do our utmost to secure a uniform system of management and nutrition over a long period of years. There is no 'deliberate' experimentation. Results are being measured continuously. So far as I am aware, nowhere else is this principle of holding environment, etc., reasonably constant being employed for dairy cattle. Yet it is precisely the same principle as that employed, though not for genetic purposes, by the most venerable of all agricultural research institutes. The 'classical' fields at Rothamsted, where certain plots have been under the same treatment for ninety years, form an excellent example of this method of passive research.

Already, and without the results of this planned and yet passive research, there is information of undoubted value, of which perhaps the most important is that quantity of milk is transmitted to a great extent independently of quality as measured by fat-yield. But many of the other results are still open to question. The problems that are now being tackled are not so much the determination of the number of genetic factors concerned in the transmission of total yield as the analysis of particular aspects of total yield, e.g., persistency of lactation. The various characteristics of the lactation must be considered in relation to each other.

In other words, we are not so much concerned with determining the number of genetic factors involved, as with analysing the lactation curve of individual animals under a standard environment. (To ensure this, the greater number of the calvings occur in two months of the year.) Two cows may give the same yield of milk, but the one may give that yield in 200 days whereas the other takes 300 : or the two cows may give the same yields in the same time but yet have widely different lactation-curves. These, therefore, must be studied with reference to the various qualities of the milk, amount of fat, sugar, protein, and natural minerals, as well as the colour, and size of fat-globule, etc., nor must the question of the relative weights of the cows be ignored both before and after the lactation. And all these are peculiarities concerning which we can already trace the workings of heredity. It is of fundamental importance to understand the action of these characteristics and their reaction on one another.

Thus it is small wonder that simple selection by the mating of the best to the best gives a large proportion of disappointing results. One cow may yield 2,000 gallons for one set of genetic reasons, whereas another cow will accomplish the same yield while possessing a very different constitution. There is, therefore, need for analysis.

Moreover, research of this nature will certainly discover whether abnormal modes of inheritance are operating, or whether quantity is simply a matter of multiple factors. These abnormal modes include such things as sex-linked

factors, and those combinations of genes which act as inhibitors of yield. The existence of such modes of inheritance can easily slow down progress by simple selection.

Further, *economic* production may actually be best obtained when genes are in the heterozygous state. Personally, I do not think that this is the case in milk-production, though there are strong grounds for believing it to hold good for meat-production. In any case this is obviously a point that must be definitely ascertained: the required knowledge can only come from experiment.

A further point is to find out whether certain combinations of characters, desirable from the standpoint of the practical breeder, are genetically possible. For instance, certain breeders desire cows giving 3,000 gallons of milk with 5 per cent butter-fat. The analysis-stage might conceivably show this to be as impossible as the desire of other cattle-breeders to establish a roan-coloured breed, roan being a heterozygote of red and white.

A definite by-product of this type of investigation is that it enables a correlation to be made between the genetical and physiological factors affecting milk-production. No gene ever works directly on the character. Manifestation of the character follows some reaction of the anatomical or physiological structure. Inasmuch as this type of experiment provides what may be called the 'genetic yield', these yields may be correlated to certain post-mortem findings, *e.g.*, to the size of the organs of internal secretion. The maintenance of a herd for genetic experiment thus provides the ideal material for research into the physiology of milk-secretion.

It is thus fundamental that, in applying the science of genetics to the complex problems of milk-production, the various genetic factors first be analysed. There can be no shadow of doubt that analysis is the pre-requisite of synthesis. Meanwhile, patience must be exercised by those who would question the value of the application of the science of genetics. Rome was not built in a day, and the Romans built with stone and not with cattle that require nine months to conceive and a further three years to produce.

With commendable reserve the United States Department of Agriculture [2] published only last year the results of the first planned experiment on the inheritance of milk-yield. It was a simple problem dealing with inbreeding. The experiment was begun in 1908, a quarter of a century previously. It has now been incorporated with a bigger experiment started about fifteen years ago, the results of which, I believe, will cause surprise to those who decry genetical experimentation with cattle on account of the slow rate of reproduction.

It can, therefore, be stated with some confidence that we now see our way round the Scylla of the disentanglement of the genetic factors. This does in part answer the question, but not wholly, for on the other side of the genetic ship

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looms up the Charybdis of the likelihood that the analysis will reveal so many genetic factors interacting with each other as to make the synthesis of the problem in its practical application almost an impossibility. An adequate reply to the opening questions of this paper demands the discussion of this point.

To those who have time to reflect, the practical outcome of the work of the plant-geneticist is little short of amazing. For many crops he has managed to make two blades grow where none could grow before. We, animal geneticists, have good cause for envy, and it is well that we should point out that this cause lies not merely in the greater rate with which one generation of agricultural plants succeeds another and the large populations which can be conveniently raised, but also in the fact that the productivity of a plant is, as a rule, infinitely more easy to measure and to assess than is the productivity of an animal.

The problem of an increase in the productivity of our live stock—and the maintenance of that increase—is not so simple as, say, the problem of an increased yield in maize. Let me refer to the work of Winter [3], which has been recently the subject of some discussion. To quote 'Student' [4], Winter 'succeeded, by continuous mass selection, in producing two races of maize one of which has more than twice, and the other less than one-third, the normal oil-content'. 'The movements of the means were, respectively, more than twelve and seven times the " inherent " standard deviation.' 'Student' estimates that at least 100 to 300 factors would be needed and considers that the actual number runs into thousands. Dealing with this point, Dr. Fisher [5] states that the experiment (which ran for twenty-eight years) is a direct demonstration that selection has the exact effects that selectionists have ascribed to it.

Can we do likewise if we breed our cattle upon the same principle of selection ? There is no doubt that selection can greatly increase the productivity of scrub stock, but as the productivity of improved stock rises so does the rate of improvement decrease. The progress becomes so slow relative to the passage of years that we must now perforce accustom our ears to the astonishing slogan of certain advisers of our farmers that it is useless to use a bull that is out of a high-producing cow, a statement based on the fact that certain bulls of such breeding do not fulfil the expectations of the owner. Nevertheless the occurrence of such animals does fulfil the expectations of the geneticist.

It is curious that some of those of our practical advisers who most decry the simple selection embodied in the ancient rule of ' mate the best to the best ' do themselves advise merely an elaboration of simple selection. So keen are these people on their slogan of the ' progeny test ' as the salvation of the British dairy industry, that they go out of their way to decry past methods of selection—combined or not with pedigree. They quite ignore the point that the progeny test is merely the logical refinement of existing methods of selection,

There can be no doubt of the value of simple selection, combined with pedigree and the progeny test as a definite means for improving the productivity of our dairy cattle. There is equally no doubt that the rate of improvement is somewhat slower than it was. Granted time, patience, and money, can we reasonably expect that selection alone will effect the desired improvement in a manner such as Winter has shown to be possible with maize?

I do not think so. Without more fundamental knowledge, the rate of improvement is bound to get slower. The problem is not the simple one of selection for one particular object. In striving to achieve a definite race of high producers, we desire to obtain a multitude of characters each of which depends upon a multitude of genes. I do not suggest that it is worth our while to determine precisely the number of genes involved in, say, an increase in the sugar-content of milk. But what is definitely of value is to discover whether an increase in the sugar-content can be secured by simple selection, and whether it is genetically or physiologically incompatible with the selection of other important characteristics. Unless we know these things, selection is bound to bring in its train a considerable amount of disappointment.

But have we as yet obtained an adequate knowledge of the pure science of genetics? He would be a fool who would so presume. Take, for instance, Dr. Fisher's theory of the evolution of dominance: with Nature as the agent of selection it is essential that those characters which benefit the organism be transmitted in a dominant manner. Dominance is, therefore, acquired by such characters, though we are ignorant of the means by which it is acquired. With man as the selecting agent, it is of decided benefit to the species that the desired characters (i.e., the productive characters of our live stock) be transmitted in a recessive manner. At present they most decidedly are not. Is it possible that just outside our sphere of knowledge there exists a mechanism for the evolution of recessivity?

But such an hypothesis as the evolution of recessivity demands systematized inbreeding for productivity. The outcome of the recently published work of twenty years' inbreeding by the United States Bureau of Dairy Industry [2] shows great possibilities in the direction of stabilizing a high yield (1,700 gallons) by this method. Success in inbreeding demands, however, a certain knowledge of the mode of inheritance of the character and particularly if sex-linkage is operating. Here is a further reason for research both pure and applied.

The demands of the market are not stable. The consumer of agricultural produce is—to the producer at any rate—fickle in his likes and dislikes. Supposing selection to be more effective than I am willing to admit, it is bound to take at least twenty years to obtain the desired type. In the meantime the taste of the consumer is sure to have changed. Not only is the taste of the consumer liable to alter in twenty years, but the general methods of production are also likely to be revolutionized by circumstances over which the

looms up the Charybdis of the likelihood that the analysis will reveal so many genetic factors interacting with each other as to make the synthesis of the problem in its practical application almost an impossibility. An adequate reply to the opening questions of this paper demands the discussion of this point.

To those who have time to reflect, the practical outcome of the work of the plant-geneticist is little short of amazing. For many crops he has managed to make two blades grow where none could grow before. We, animal geneticists, have good cause for envy, and it is well that we should point out that this cause lies not merely in the greater rate with which one generation of agricultural plants succeeds another and the large populations which can be conveniently raised, but also in the fact that the productivity of a plant is, as a rule, infinitely more easy to measure and to assess than is the productivity of an animal.

The problem of an increase in the productivity of our live stock—and the maintenance of that increase—is not so simple as, say, the problem of an increased yield in maize. Let me refer to the work of Winter [3], which has been recently the subject of some discussion. To quote 'Student' [4], Winter 'succeeded, by continuous mass selection, in producing two races of maize one of which has more than twice, and the other less than one-third, the normal oil-content'. 'The movements of the means were, respectively, more than twelve and seven times the " inherent " standard deviation.' 'Student' estimates that at least 100 to 300 factors would be needed and considers that the actual number runs into thousands. Dealing with this point, Dr. Fisher [5] states that the experiment (which ran for twenty-eight years) is a direct demonstration that selection has the exact effects that selectionists have ascribed to it.

Can we do likewise if we breed our cattle upon the same principle of selection ? There is no doubt that selection can greatly increase the productivity of scrub stock, but as the productivity of improved stock rises so does the rate of improvement decrease. The progress becomes so slow relative to the passage of years that we must now perforce accustom our ears to the astonishing slogan of certain advisers of our farmers that it is useless to use a bull that is out of a high-producing cow, a statement based on the fact that certain bulls of such breeding do not fulfil the expectations of the owner. Nevertheless the occurrence of such animals does fulfil the expectations of the geneticist.

It is curious that some of those of our practical advisers who most decry the simple selection embodied in the ancient rule of ' mate the best to the best ' do themselves advise merely an elaboration of simple selection. So keen are these people on their slogan of the ' progeny test ' as the salvation of the British dairy industry, that they go out of their way to decry past methods of selection—combined or not with pedigree. They quite ignore the point that the progeny test is merely the logical refinement of existing methods of selection.

There can be no doubt of the value of simple selection, combined with pedigree and the progeny test as a definite means for improving the productivity of our dairy cattle. There is equally no doubt that the rate of improvement is somewhat slower than it was. Granted time, patience, and money, can we reasonably expect that selection alone will effect the desired improvement in a manner such as Winter has shown to be possible with maize?

I do not think so. Without more fundamental knowledge, the rate of improvement is bound to get slower. The problem is not the simple one of selection for one particular object. In striving to achieve a definite race of high producers, we desire to obtain a multitude of characters each of which depends upon a multitude of genes. I do not suggest that it is worth our while to determine precisely the number of genes involved in, say, an increase in the sugar-content of milk. But what is definitely of value is to discover whether an increase in the sugar-content can be secured by simple selection, and whether it is genetically or physiologically incompatible with the selection of other important characteristics. Unless we know these things, selection is bound to bring in its train a considerable amount of disappointment.

But have we as yet obtained an adequate knowledge of the pure science of genetics? He would be a fool who would so presume. Take, for instance, Dr. Fisher's theory of the evolution of dominance: with Nature as the agent of selection it is essential that those characters which benefit the organism be transmitted in a dominant manner. Dominance is, therefore, acquired by such characters, though we are ignorant of the means by which it is acquired. With man as the selecting agent, it is of decided benefit to the species that the desired characters (*i.e.*, the productive characters of our live stock) be transmitted in a recessive manner. At present they most decidedly are not. Is it possible that just outside our sphere of knowledge there exists a mechanism for the evolution of recessivity?

But such an hypothesis as the evolution of recessivity demands systematized inbreeding for productivity. The outcome of the recently published work of twenty years' inbreeding by the United States Bureau of Dairy Industry [2] shows great possibilities in the direction of stabilizing a high yield (1,700 gallons) by this method. Success in inbreeding demands, however, a certain knowledge of the mode of inheritance of the character and particularly if sex-linkage is operating. Here is a further reason for research both pure and applied.

The demands of the market are not stable. The consumer of agricultural produce is—to the producer at any rate—fickle in his likes and dislikes. Supposing selection to be more effective than I am willing to admit, it is bound to take at least twenty years to obtain the desired type. In the meantime the taste of the consumer is sure to have changed. Not only is the taste of the consumer liable to alter in twenty years, but the general methods of production are also likely to be revolutionized by circumstances over which the

British farmer has no control. Unless we have fundamental knowledge concerning the inheritance of the characteristics of the lactation, we need not hope to be able to keep pace with market fluctuations.

Pending adequate analysis of the problem, a continuance of existing selection methods (with the re-enforcement of the recent remarkable rediscovery of the progeny test) is much to be desired and will, on the whole, give good results. When, as is bound to happen, there is a popular reaction to the progeny test—not because it has failed to give results, but because those results have not been as great as the advocates of the test are now promising—than the science of genetics will be able to make a further and definite contribution to the subject, provided that the foundations for such work have been well and truly laid.

Furthermore, the productivity of our live stock depends upon the close interrelation of the control of disease with nutrition and genetics. The Scottish shorthorn was not the product of Amos Cruickshank of Sittyton. It was the product of Cruickshank and turnips and straw—all three of them from Aberdeenshire. Likewise, the Aberdeen-Angus was not the product of McCombie, but of McCombie, turnips, straw, and oil-cake. As the biologists and farmers of Aberdeenshire fully appreciate, the science of the nutrition of our live stock is making great headway. But it cannot outstrip the genetic application. Improved methods of feeding put new stresses on the machine that can only be met by the adjustment of the hereditary constitutions of the animals. At present we lack the basal knowledge necessary to effect such alterations which the future will certainly demand.

Let me illustrate this. Hitherto the 'basal ration' has been a most useful conception in the theory of feeding live stock. In Edinburgh, we have two strains of pigs. For the one, the average consumption of meal, per lb. of liveweight gain, is over 4 lb.; for the other, the economical pigs, it is only about $2\frac{1}{2}$ lb. The same holds good for cattle, both for milk and beef. Thus, by genetical methods, the interior economy of an animal can be modified to suit nutritional requirements.

Finally, to those critics of the genetical method for the improvement of our dairy cattle, I would say: It is worthy of remark that the hereditary improvement in the yield of our dairy cows has taken place in the post-Mendelian era. The fact that the breeding of live stock has, in part, been reduced to a science, has clarified thought and put the practice of live stock improvement upon a logical basis. Moreover, each new generation of breeders has no longer to disentangle fact from fancy. The existence of the science enables the young breeder to start at the place where his father left off. This is no mean achievement to the credit of the science of genetics, for it must be remembered that, whereas, the improvement of the hereditary qualities of crops rests safely in the hands of a few skilled research workers, the improvement of our live stock rests with the innumerable breeders of that live stock distributed throughout the world.

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THE INHERITANCE OF PRODUCTIVITY IN FARM LIVE STOCK

III. BREEDING FOR EGG-PRODUCTION

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Discussing the future possibilities for increasing egg-production in poultry, Jull [1] has stated that progress largely depends upon the poultry-breeder's ability to control heredity. Further, he suggests that " heredity can be controlled and directed best only when the knowledge of poultry-breeders develops sufficiently to enable them to select breeding stock that will transmit to their offspring the most desirable qualities. Selection is the keynote in the programme of future development."

Selection without an exact knowledge of the mode of hereditary transmission of desirable qualities, but with a belief that they are transmissible from parent to offspring, has been the basis of all breeding practices in the past. It has been responsible, together with improvements in husbandry, for the tremendous increase in the production records of our modern domesticated breeds when compared with the ancestral types from which they have sprung. Such advance bears witness to the importance of heredity.

In attempting to assess the possible value of the science of genetics to the cause of increased fecundity in poultry, we should begin by considering the results that are now obtainable when selection along certain definite lines is practised. An examination of the production figures at egg-laying contests testified to the skill of the intelligent breeder in developing highly fecund strains of birds. His methods of selection, however, when improved flocks are dealt with lead to negligible progress over a fairly long period of time. This has been demonstrated by Dunn [2] from an analysis of egg-laying contest figures in America. Over a period of nine years he found that there was little material change in average egg-production. This is very significant. Since only a small percentage of a flock of birds is rigorously selected by certain standards for participation in egg-laying trials, it follows that the results obtained represent *practically* the highest individual performances possible with the methods of selection now used by breeders. The production of the flock as a whole, however, usually falls far short of these individual records, and perhaps the most pressing question to be faced from the economic stand-point is that of devising a means of raising the figure for average production, even in the most improved flocks.

The problem of improving egg-yield by breeding has been the incentive to much scientific work, which has taken the form of analytical attempts to define and measure the desirable qualities that a hen should possess in order

that maximum production may be attained.   From observation and experiment Goodale and Sanborn [3] conclude that there are, at least, five desirable qualities which, if present in a bird, lead to high annual production.   These are :—

1. Early sexual maturity.
2. High intensity of production.
3. No winter pause in production.
4. Non-broodiness.
5. High persistency of production.

Even a casual acquaintance with the hen reveals that for certain periods in her reproductive life, egg-production ceases for varying lengths of time.   The most definite gap in egg-laying occurs during the time when the old plumage is being discarded and the new feather-covering is growing in.   Since this is a characteristic annual phenomenon, typical of the vast majority of birds, it will be seen how important its effect is upon the annual-production record of the pullet — a figure which is arbitrarily fixed as the number of eggs laid in the 365-day period from the time when she produces her first egg.   Since the moult is very variable, both in point of time and duration, it is obvious that the annual production increases in proportion as the onset of the moult is retarded and approaches the end of the 365-day period.   At the Institute of Animal Genetics, Edinburgh, we have several hens among our flock that, over a two-year period, have failed to undergo the moult, and egg-production has not ceased during this time.   This is the ideal persistency.

High intensity means that a bird must be persuaded to lay as often as possible within the prescribed period.   "Winter clutch size", *i.e.*, the average number of eggs laid in succession up to March 1, is used as an indicator of intensity of production, because it has been found to be significantly correlated with annual egg-production.

Most pullets are hatched so that they become sexually mature in the autumn. It is rarely found, however, that continuous production occurs throughout the winter months without the appearance of at least a few successive days on which no eggs are laid.   A break in the continuity of production of this magnitude classifies the bird as exhibiting "winter pause".

Another physiological manifestation that affects the ultimate performance of a hen is that of broodiness.   During this phase of maternal expression, egg-production ceases for a variable length of time, and is recommenced only after the signs of broodiness have waned.   This is a problem which affects particularly that branch of the industry concerned with the heavy breeds of poultry ; broodiness is a comparatively rare phenomenon in leghorns, for example,

Recorded observations of many workers have shown that quick-maturing pullets tend to make the highest annual records, and this emphasizes the necessity for considering early sexual maturity when increased records are desired.

These five qualities are, perhaps, obvious ones to select for, if an increase in egg-yield is desired ; it will be interesting to see what amount of improvement may be obtained in a relatively *unimproved* flock by continuous selection along these lines. Fortunately, the results of two separate experiments are available, one carried out by Marble and Hall [4] and the other begun by Goodale and continued by Hays and Sanborn [5] (Table I).

TABLE I

Marble and Hall, 1930	Goodale, Hays and Sanborn, 1934.
Leghorns	*Rhode Island Reds.*
Average production increased from 118 to 196 eggs	Average production increased from 134 to 222 eggs.
Percentage increase 66·1	Percentage increase 65·6.

It is not suggested that such a rate of improvement could be obtained in the best flocks by means of selection based on the five qualities outlined above, because in all probability the higher averages produced now-a-days have resulted from an unconscious use of these qualities as a basis of selection over a comparatively long period of years. Both experiments started with birds that did not give a very high average egg-yield, and continuous selection for increased egg-production was maintained over a period of twenty years. The results obtained in both sets of experiments showed a remarkable similarity in the amount of increase in the average yield, and this is the more striking when it is considered that not only were different breeds used, but that the initial egg-production of the two flocks was also different. From these experiments it is possible to deduce that the methods employed are along the right lines but the question has still to be faced as to what is the final degree of improvement in egg-yield that may be obtained by these methods.

From an analysis of their flock records for the years 1928 to 1932, Hays and Sanborn [5] show that whereas, over 50 per cent of their birds exhibited three or four of the desirable characteristics, and 16 per cent all five of them (Table II), this improvement was only about one-sixth of that theoretically anticipated, for there is no known reason why all the birds cannot carry all five desirable characteristics.

TABLE II

Relation of Number of Desirable Characters to Annual Production

Characters Number				Birds Number	Birds Per cent of total	Egg-production. Average per bird
0	.	.	.	31	1·3	149
1	.	.	.	158	6·9	157
2	.	.	.	375	16·3	174
3	.	.	.	717	31·2	201
4	.	.	.	648	28·2	227
5	.	.	.	371	16·1	252

That these particular desirable characters are inherited has now been established and a reasonable idea of their mode of inheritance has been gained.

Early sexual maturity apparently depends on two independent dominant genes, either of which can produce, for example, the onset of egg-laying in the Rhode Island Red breed before the hens are 215 days old. One of these genes is sex-linked, the other autosomal (Eo and E'E').

For high intensity of production, also, two dominant genes are necessary (II and I'I'). Hens carrying gene 1 have alone an average winter-clutch size greater than 2, but less than 2·6. Gene 1' gives a clutch size of about 2·6. Both genes together increase the clutch size up to 3 or more eggs throughout the winter season. Both genes are autosomal and cumulative in effect.

For winter pause there seems to be only one dominant gene, M. Desirable birds therefore carry the recessive form, mm.

Broodiness has been found to be inherited on a two-factor basis. Two dominant complementary genes, A and C, are necessary to produce it. There is no evidence of sex-linkage and non-broody birds are of three general classes : (1) those lacking both A and C ; (2) those carrying A and lacking C ; and (3) those carrying C and lacking A. The latter two classes make it evident why broodiness is so difficult to eliminate completely from a flock, since females of these constitutions, though not exhibiting broodiness themselves, will produce broody offspring if mated to males carrying the complementary gene they lack.

The data for persistency indicate that it is inherited on the basis of a single dominant autosomal gene, P.

L

So far as experimental work has shown, the ideal hen should have a genetic constitution with regard to the desirable characters leading to high egg-production, as follows : Eo, E'E', II, I'I', mm aacc, PP.

It will readily be seen why progress by methods of selection is apt to be extremely slow, for there are possible in a flock, 65,496 * genetically different classes of birds with regard to the five characters involved, and only one of these would give fixity of type. Again, since five of the eight genes, from which these characters originate, are dominants, distinctions between their heterozygous and homozygous forms are small, and at present apparently undetected, so that if, as must occasionally occur, a hen homozygous for all the desirable qualities should arise, the chances of its being recognized as such are extremely small. In the case of the male, the difficulties are further increased by the fact that, though for true-breeding stock he must carry the desired characters in the homozygous form, yet he cannot in himself exhibit them.

It can well be imagined then that the chances of producing a strain with constant genetical constitution is extremely remote when selection methods alone are practised ; and it is indeed doubtful whether much further progress would be made in the best flocks, although considerable progress is possible in un-improved stock.

If we refer back to the original statements of Jull that ' the possibility of increasing egg-production depends on the poultry breeder's ability to control heredity ', and ' that selection is the keynote in the programme of future development ', a certain amount of confusion arises. Even with the knowledge of the mode of inheritance of the necessary qualities leading to high annual egg-production, it cannot be said that a policy of selection along these lines owes anything to the fact that a genetical analysis has been made. In fact, it may be said that ignorance of the genetical work should produce about the same amount of improvement in a flock, because the qualities selected for are just those which, if inherited, would tend to increase production. So far, the service of genetics has been to prove what has hitherto been a belief, *viz.*, that certain desirable qualities are inherited in a definite and orderly manner.

Unless we are to agree with Babcock and Clauson [6] that the real service of genetics to animal breeding lies in promoting clarity of thought, the geneticist must show how the knowledge gained by experiment can best be used to improve existing breeding practices. In this particular field he must show the breeder how he can control heredity.

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* This figure is taken from Hays' publication but is obviously incorrect. The number of genetical classes possible when dealing with eight genes, only one of which is sex-linked, would be 4,374 when females alone are considered, and 6,561 if males are considered : a total of 10,935.

The real limitation of selection lies in the fact that, owing to the apparent similarity of production of the homozygous and heterozygous gene groupings, it is impossible to forecast with accuracy the productive capacity of all offspring from any mating. To illustrate this with regard to one particular quality, say persistency, making use of genetical knowledge, let us suppose that only females showing persistency were selected as breeders; they would have either the genetic constitution PP or Pp. There could be no evidence from the male himself as to his genetic constitution, and so, from matings with persistent females, there are three groups, each of two classes, into which his resultant progeny might fall (Table III).

TABLE III

Parents Offspring

Group 1—

| PP♂ × PP♀—PP | . | . | . | all persistent and true-breeding. |
| PP × Pp—1PP : 1Pp | . | . | . | all persistent, but only half true-breeding. |

Group 2—

| Pp♂ × PP♀—1 PP : 1 Pp | . | . | all persistent, half true-breeding. |
| Pp × Pp—1 PP : 2 Pp : 1 pp | . | . | three persistent to one non-persistent, but two in three of persistent not true-breeding. |

Group 3—

| pp♂ × PP♀—Pp | . | . | . | . | all persistent, but not true-breeding. |
| pp × Pp—1 Pp : 1 pp | . | . | . | one-half persistent, but not true-breeding. |

The fact that females exist in the stock which exhibit persistency, and yet cannot transmit this quality to all their offspring, makes it extremely unlikely that, using this as a basis of selection, a strain homozygous for the character would ever be developed, even though care was taken to breed only from birds all of whose progeny were persistent. Such a procedure could only tend to limit the selection of breeders to the first three classes, and since only the first is of fixed constitution, a certain number of undesirable, *i.e.*, recessive individuals would almost inevitably occur among the succeeding progenies, which might belong to any of the first four classes.

Genetical work has given us at least one clear conception, namely, that a bird may exhibit a desirable character, but only be able to transmit it to part of its offspring. The immediate application of this knowledge could only be made if further experiment were carried out in sufficient detail to demonstrate whether measurable differences between the hetero- and homo-zygous forms exist. If this could be done, then the development of homozygous strains carrying all the desirable characters, could be rapidly undertaken. Only by this means can the control of heredity by breeders be readily visualized.

On the other hand, should this information be unattainable, that is, supposing measurable differences in egg-production cannot be found between hetero- and homo-zygous forms, the geneticist still has a method of attack that would eventually lead to the analysis of the genetical constitution of any bird. In the first place, it would be necessary to build up a stock containing all the homozygous *recessive* forms of the characters to be investigated. This could be done by selection much more easily than by the reverse process. The value of selecting for recessive characters is well illustrated by the results of selection against broodiness ; this has been almost completely eliminated from many breeds of fowl, and even in breeds like the Rhode Island Red, where broodiness is of common occurrence, the value of selection against it is shown in the figures of Hays and Sanborn, where the percentage of birds exhibiting this characteristic phenomenon dropped from 86 in 1916 to 12 in 1932. Theoretically then, the building up of a strain of birds homozygous for the recessive characters should not offer great difficulty. Such birds would have the following genetical constitution with regard to the characters selected against : eo, $e'e'$, ii, $i'i'$, pp, aa cc, (late maturity, low intensity, low persistency of production and non-broodiness). The only character of the group that would offer any difficulty would be winter pause, which depends on a single dominant gene. The recessive homozygous form could not be expressed in such a strain of birds because of the activity of the other genes selected to give minimum production ; this hindrance could be overcome, however, by first of all fixing this character in the stock from which the homozygous recessive strain would subsequently be developed.

Having once obtained such a strain of birds, it would be possible to determine accurately, and well within the productive life of the bird, the genetical constitution of any male or female, by means of matings with the recessive stock. The desirable qualities, which combine to produce high egg-yield, are most probably dependent on the same genes in the different varieties of the domestic fowl, so that, it would not be necessary to obtain testing strains for all the different breeds of fowl.

Before closing this discussion of the inheritance of egg-production, it is necessary to emphasize the fact that the survey of the problem has been made from the simplest possible genetical angle. We have dealt only with the behaviour of certain inherited characters without suggesting factors that might intervene to complicate breeding practice, and so we have omitted to stress the important rôle played by environment and its effect on the expression of the gene. It is obvious, however, that this type of reaction also falls within the province of the geneticist—to determine how the expression of the genotype may be modified under varying environmental agencies. It goes without saying, for instance, that nutrition plays a most important part in deciding, whether or not, the full genetic capabilities

of an animal are to be expressed. Apart from nutrition, perhaps the most striking effect on egg-production derived from control of extraneous environmental influence is that which follows increased exposure to light.

Just as we have a measure of the intensity of inheritance from observations on parent and offspring in breeding practice, so to determine quantitative effects of environmental factors on gene expression, it is necessary to have stock whose genetical constitution is known.

There is yet another phase of genetical inquiry that may perhaps be considered of mainly academic interest, but from which we now have indications that it may lead shortly to more practical and less tedious methods of controlling heredity than that outlines earlier in this paper. In the final genetical analysis the gap in our knowledge which exists between the presence of the gene, on the one hand, and the exhibition of its end-product—the character—on the other hand, must be bridged.

Hammond [7] has given us a suggestion as to the type of mechanism that may lie between the genes and their characters. In the experiments on the increased productivity due to an increased light ration, it is his opinion that light acts by stimulating the anterior pituitary gland to increased secretion, and this substance circulating in the blood stimulates in turn the ovary to increased production. He further suggests that the level of this substance in the blood may explain the difference between high and low egg-producing strains and breeds.

It would be interesting to determine which of the five qualities outlined previously as leading to increased egg-production are likely to be affected by this stimulus.

Early sexual maturity.—The relation existing between sexual maturity and the anterior lobe of pituitary is too well known to need labouring, but the work of Domm [8] in Chicago merits a brief notice. By suitable injections of a preparation of this gland, he was able to produce precocious development in the sex glands and secondary sexual characters of fowls, so that some of his male chicks were crowing nine days after hatching, and began to tread when only a fortnight old.

Persistency.—The length of time a bird continues laying after the production of her first egg is limited by the time of appearance of the moult. In one of our birds we were able to eliminate the moult by implanting anterior pituitary glands from other birds (Greenwood [9]).

Broodiness.—From extracts prepared in our laboratory, Professor F. A. E. Crew has been able to produce the typical behaviour of broodiness in Leghorn fowls, a breed in which it rarely occurs. Even young hens reacted to the stimulus before sexual maturity was reached.

Intensity and no winter pause.—Gutowska [10] has shown that oral administration of anterior lobe of pituitary leads to an increase in egg-number. There was an increase in the number of follicles in the ovary, and also an increase in their size. The results were most striking in the early part of the year (before spring).

Finally, Parkes [11] has shown recently that the removal of this gland leads to the atrophy of the sexual glands. I have purposely avoided entering into too much detail with regard to these extremely significant observations, since at the present time control of heredity by these means remains still in the realm of possibility and has hardly reached the fringe of probability. An immense amount of work still remains to be done.

SUMMARY

1. Selection methods practised along the right lines tend to increase productivity, particularly in unimproved stock, but progress is slow in improved stocks because of the inability of the breeder to control heredity.

2. It has been shown that the desirable qualities to select for are inherited and that their mode of transmission from parent to offspring has been determined.

3. Such knowledge need not necessarily affect selective breeding practice because of the present inability to distinguish between hetero- and homo-zygous forms of these genes.

4. The application of genetical knowledge to breeding requires either a method of distinguishing these forms by their production records, or an accessible technique whereby the genetical constitution of an animal may be accurately determined. With this knowledge at disposal, the fixation of desirable characters in a flock can be readily made.

5. The field of work of the geneticist covers not only the mode of transmission of characters under optimum conditions, but also the effect of variations in the environment on the resultant expression of gene action. For this it is essential to deal with animals of known genetic constitution.

6. The final phase in the genetical analysis concerns the relation between the gene and the mechanism by which the end result—the character—is produced. The possibility of the control of heredity through the control of physiological processes is foreshadowed.

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THE INHERITANCE OF PRODUCTIVITY IN FARM LIVE STOCK

IV. WOOL

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The general title of these papers suggests a wider field for consideration than that simply of the inheritance of wool-characters, which has been reviewed recently by Miller [1]. In the first place, it is to be remembered that the fleece, as a covering during life, contributes to the hardiness of the animal in its ability to survive the rigours and changes of environment, and is, therefore, concerned in the other activities of the sheep, for meat-production, for breeding surplus stock, in some countries for milk, and so on. It is an agent, though indirect, in promoting the efficiency of the sheep as a producer of those commodities which the sheep-breeder hopes will be profitable to him. Herein it gives rise to problems in productivity rather remote from those occurring in the other animal products dealt with in these articles.

Fibre-growth is a more intimate function of the sheep's activity than is, say, milk-production of the cow's and egg-production of the hen's. It begins in early foetal life and ends only at death. There would thus appear to be three different primary sets of environmental conditions to which it is exposed, *viz.* (1) the relatively constant foetal environment, during which, however, the follicles *inter se* are affected by the sequence and density of their development [2], (2) the early post-natal, representing a change in life-habit towards (3) the post-weaning, quite independent life, in which the animal attains maturity, becomes a functional ruminant, and so exposed to all the exigencies of nutritional environment incidental to free-grazing animals.

The changes that occur with general development are obvious in many breeds. Many Merino lambs are born hairy; the birth-coat hairs are shed or lost, and leave the growing lamb with a fleece of practically pure wool. The lambs of some of our Down breeds, especially the Suffolk, are more or less darkly coloured at birth; by shedding and replacement, the relatively pigment-free adult coat is acquired; from maturity onwards we find other changes in fleece character. Recent work on time or age changes, from birth to maturity is pervaded with the idea that definite relationships exist between natal and adult coats, and that selection for desirable adult fleece-characters can be exercised early in life [3]. It is clear that not only is the follicle-population variable in time, but also the forms of the fibre-product are alterable.

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Throughout the adolescent and mature phases the changes in fleece-growth that can follow alterations in nutritional level are very varied, and may be of some magnitude. For example, in one case, which has been studied, of an Australian Merino fleece, it was estimated that in terms of average fibre-volume per month, the rate of production suddenly accelerated by over 100 per cent at one period of the year [4]; and the wool-fibres are not the only products of the skin-and-follicle system which make up the growing fleece [5].

But so far as wool-productivity is concerned, we are mainly involved in considering wool as an annual crop of some direct value to the farmer. For the most part its course to the real consumer is peculiarly tortuous and prolonged, and through the innumerable mixings which occur the individual fleece loses all its identity. The cash value of the wool crop to the farmer is ordinarily some function of the weight of the raw wool, the proportion of grease and other non-wool materials in the raw fleeces, and the estimated fitness of the amount of actual pure wool present for particular manufacturing purposes. These three factors are very variable, from one animal to another, from flock to flock between different breeds, and according to locality and husbandry. Moreover, when the wool-using industry is viewed, as a whole, it is readily appreciable that absolute uniformity of the supply of raw material is not desirable of the inherent attributes of the wool-fibres in the fleece which affect its manufacturing utility, length, soundness, 'quality' or fineness, uniformity, fineness to its particular type or breed, and general character, may be mentioned, but of these only length and fineness are readily measurable, and have, therefore, been studied most.

The most useful combination of these characters from the manufacturer's point of view is not necessarily that which the farmer would consider optimal, quite apart from other attributes of the fleece with which the latter is concerned. For example, fleece-density, in part contributory to fleece-weight, is of more direct interest to the breeder than to the user, in so far as it is involved in the rain-coating action of the fleece. Yet it is apparently not always dissociable from the length-fineness complex.

In view of such interrelations, it is perhaps not surprising that the majority of investigators conclude that apart from questions of colour, the inheritance of most fleece-characters is dependent upon multiple factors.

Apparently conflicting results have, however, been noted. For example, whilst Hill [6] found the F_1 intermediate in wool-fineness in Hampshire-Rambouillet crosses, Davenport and Ritzman [7] noted that the F_1 approached the *coarser-wooled* Hampshire parental type; yet these investigators also describe intermediate F_1s in crosses involving South-down and Oxford rams and Rambouillet ewes with a tendency to approach the *finer-wooled* parent. At the same time, they record in the Oxford cross an F_1 wool-length type, whilst in the South-down and Hampshire crosses, the F_1 had longer wool than either parental form. Observations on Scotch Halfbred (Border Leicester ram × Cheviot ewe) also

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indicate partial dominance of the longer wool, but if amount of fibre (*i.e.* volume) is considered, then these conclusions may have to be modified, since in this cross the F_2 average fibre-volumes tend to swing towards the Cheviot parental type rather than the Border Leicester [8].

If we admit that multiple-factor systems determine fleece-character inheritance, two important considerations arise. The first is the apparent futility of searching for single genetic factors which may override or profoundly affect the fleece-complex of wool characters. Miller [1] has raised this point, but re-emphasis is not out of place. The second concerns methods and degrees of selection. The suggestion can be made that some of the divergent results of investigations are explicable on the grounds that one parental form has been selected more rigorously, or is less heterozygous, for one particular fleece-character and exhibits a degree of prepotency, just as in ordinary breeding practice appear strains or individuals decidedly impressive. Conceivably, such an interpretation may be applicable, for example, in the first and second generations of the Border Leicester-Cheviot cross, which show bimodal frequency distributions of fibre-length similar to those found in the Border Leicester [8].

Selection for wool-productivity is in practice pursued within the single breed or flock ; in ordinary husbandry its objects are generally to reduce the variability of fibre-character on each animal and from one animal to another, *i.e.* within the flock or breed, as well as to improve performance in some desired directions, such as greater density, higher fleece-weight, better yield or longer staple. Outstanding examples of the progress of such selection are to be found among the ' stud ' Merino flocks, and from them we can draw conclusions, not only as to the methods of improvement, but also as to the ways in which the geneticist may accompany the practical breeder.

In multiple-factor situations, the phenotype closely reflects the genotype. In the stud flocks, we find that whereas first selection may be phenotypic, the progeny test is widely applied, and the method of breeding involves a progressive infiltration of a genotype throughout part of the flock. Thus, briefly, a stud ram will be mated to a few selected stud ewes, some of his ram offspring will be mated to other stud ewes, and so on, until, when proved, his influence can be applied where necessary in the flock. Even in the larger commercial flocks, some selected ewes are retained almost solely for use with introduced rams, to breed rams for general service, forming a ram-testing and breeding flock within the main flock. But the function of the studs, or pedigree flocks, is to supply so-called ' flock rams ' for use elsewhere, which means, in many different environments. The breeders realize the importance of the extra-ordinary effects of environment on the animal and its fleece ; hence a certain variability of genotype is deliberately maintained so that the different demands for flock rams may be met. To this end the variability is kept possibly relatively greater among the top stud animals than among the flock ewes.

But in surveying the wool-producing countries, one cannot help being impressed by the remarkable way in which certain localities are pre-eminent for selection and improvement, constituting in effect centres for the breed from which emanate stock for commercial use in other areas. The type most successfully or most readily developed in one breeding centre is, however, not necessarily very like that from another. Further, they do not perforce react in precisely the same way when transported to yet another environment.

A few instances from the many may be quoted. In the British Isles, is a multiplicity of breeds, almost every one possessing a territory within which it either predominates or produces stock for crossing, and there are also local variations in type of practical importance. In Australia, among the Merinos, are the South Australian, the Riverina, and other New South Wales forms of strong wools ; stocks of strong or medium wool origin exhibit different characters when pastured in fine wool areas, whilst among the fine wools, distinction can be made, for example, between the Tasmanian, Victorian, and New South Wales types. Parts of the Karroo areas in South Africa form fountain heads of improved breeding stock for use in other regions of the Union, and in New Zealand, among the Romneys, different localities apparently demand different strains.

In all cases it is, or should be, the general balance of fleece-characters that is borne in mind. Only a few days ago, discussion with a New Zealand breeder revealed that he had been attempting to increase his fleece-weights by selecting for greater staple-length, but he had found that at a certain stage the wool suffered by the tips of the staples becoming more weathered and wasted. Thus, it was better to sacrifice the extra length and weight for soundness throughout the staple.

All these considerations add force to the principle that the pre-requisite for selection is that environment which allows the clearest expression of genotype. In the field of wool-productivity, to aid in the recognition of genotype and to gain accurate knowledge of phenotype to which it gives rise in other environments, are objectives which the zoologist may well pursue.

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THE INHERITANCE OF PRODUCTIVITY IN FARM LIVE STOCK

V. DISCUSSION OF PRECEDING CONTRIBUTIONS

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Clarity may be served by discussing these four papers from two stand-points: firstly, the inheritance of productivity as a scientific fact, or series of interrelated facts, and secondly the methods of using those facts and interrelations most efficiently to advance the aims of animal breeders. Efficient application of fundamental knowledge to the solution of present problems may not automatically follow the discovery of that knowledge. Research in methods of application may be quite as necessary as research in discovery of the principles themselves.

So far as concerns the inheritance, each paper has stressed the complexity of the topic. It seems certain that for each trait there are many genes involved. No doubt these genes interact with each other and with the environment in many ways besides the simple additive way that may be expressed most readily in generalized formulae.

Each paper has also stressed the necessity for keeping the animals in an environment that would permit differences in productivity to develop, although the philosophical explanation for this necessity varies among the speakers. Dr. Hammond holds that the genes change adaptively in direct response to the environment, whereas most of us hold that such gene-changes have not been demonstrated experimentally, even by the many experiments expressly intended to find them, and, moreover, are not necessary to explain the facts, either of animal breeding or of evolution. Hence, we would argue that there is no necessity for invoking such a supposed direct effect of environment of genes, even as a working hypothesis. The necessity for keeping animals under an environment that will permit them to show their differences in productivity, arises from the fact that conscious selection for such differences is possible only when and as far as such differences are permitted to develop to a recognizable degree.

The sharp line which Dr. Hammond proposes between 'developmental' and 'mutational' characteristics will not, I think, be accepted as valid by many workers, either in animal breeding or in more classical branches of biology. Most of us would hold that all characteristics are developmental, depending for their full expression upon the interactions of the genes with each other and with environment as well. From this standpoint, highly hereditary characteristics are those in which most of the variance we ordinarily see, is due to differences in the genes

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which different individuals have, whilst faintly hereditary characteristics are those in which most of the variance ordinarily occurring is due to differences in environment to which different individuals have been exposed. Characteristics may further be classified according to whether the hereditary portion of the variance is due to few factors and shows sharply discontinuous classification, or many genes are involved and yield a practically continuous series of phenotypes. From this standpoint the differences between highly hereditary and slightly hereditary, or between continuous and discontinuous, characteristics, although highly important practically, involve no differences in fundamental principles, but only differences in the number of genes involved, or in the proportions of the total observed variance resulting from genetic differences and from environmental differences.

So much for the fundamental facts concerning the inheritance of productivity. Before applying those facts to breed higher productivity into a breed or strain of animals, as an engineer would apply the fundamental principles of physics and mechanics to such a task as the building of a bridge, measurements are needed of the magnitude of the forces involved, just as the engineer would need measurements of the loads his bridge was expected to carry, the amount and seasonal distribution of the water-flow which must move down the stream under it, the number of years before it is expected to become obsolete, the amount of money available for construction, etc. I venture the opinion that, in general, this is the least explored field of applied genetics, and that neglect to measure and integrate properly these variables is the major cause for the mistakes and unsound proposals that are sometimes made even by those whose knowledge of biological fundamentals is not seriously deficient.

It will not have escaped notice that all four speakers have mentioned selection as a first step in breeding for productivity, although they differ considerably in their optimism as to its *sufficiency*. I would here call attention to only two aspects of the question of how intense selection can be, even in the idealized case where the man doing the selecting is possessed of complete knowledge of the genotypes of his animals. The first is that natural fertility and longevity set serious limits to the intensity of the selection which may be practised. Each parent must eventually be replaced by another, even in a population static in size. What proportion of the female offspring must be saved for replacements ? What proportion of the males ? Here is a whole field of what we might call ' the vital statistics of farm animals ' which is as yet only slightly explored. In dairy cattle, about 50 to 60 per cent of the heifers must be saved, merely to replace their dams, which are being lost by disability, old age, and other causes of death. In swine, perhaps 10 per cent of the gilts would be enough ; perhaps 7 or 8 per cent would be enough for exceptionally well-managed herds. In chickens, perhaps 2 per cent of the eggs that might possibly be hatched into pullets would be enough for replacements. These figures set serious and varying limits to intensity of selection in the first place. One must begin with the material available. There is small use to talk

of the advantages of selecting breeding-stock homozygous for high productivity if less than 1 per cent of the population is of that genotype, and if one is forced by reproductive rates to save 10 or 50 per cent of the whole population for breeding.

The second point concerning the intensity of selection is that such intensity is weakened (much more than is generally realized) by the inclusion of more and more items in the ideal; that is, by considering many different characteristics in making the selections. In the idealized case, where n independent, equally important characteristics are to be considered in the selections and x is the proportion of the population which must be saved for replacements, the selection intensity for each characteristic singly is the same as if the nth root of x were the proportion which must be saved. Thus, if one needs only to save one-tenth of the offspring, but pays equal attention to eight independent characteristics, the intensity of selection for each such characteristic is no greater than if attention has been paid to the characteristic alone, but it had been necessary to save three-fourths of all offspring born. This, I think, is the only general basis of real antagonism between breeding for production and breeding for ' fancy points ' namely, that each additional point considered must necessarily weaken the selection which might otherwise have been practised. Nevertheless, the practical breeder *must* pay attention to several things, even though he understands perfectly this weakening effect.

Besides estimating how intense his selections can actually be, one needs (if he is to estimate the outcome at all accurately) to know first what portion of the observed variance is genetic in the narrow sense, which includes only those gene-combination effects that can be expressed by some additive scheme; second, what portion of the variance is due to gene-combination effects that cannot be expressed additively; and third, what portion of the initial variance is purely environmental in origin. Of course such a division is not absolutely correct to the last detail, since in actual practice there will be interactions between the three kinds of variance. For instance, some genotypes may be more plastic to environmental influences than others. If that be the case, and if the breeding system increases the frequency of the more easily influenced genotypes, then the environmental portion of the variance will likewise increase, although the environment does not change. If extreme refinement is important, and if the necessary data are available, a more detailed division may be made specifying how much variance is due to such interactions. However, in most practical problems, such refinements will usually produce but small gains compared with the considerable amount of approximation still remaining. Usually, the first approximation given by dividing the variance into the three parts named above will be sufficient for the purpose of practical application.

Only the genetic variance that can be expressed additively is subject to simple mass selection. The refinements of selection methods, such as the progeny or the judicious use of pedigree information, serve mainly to reduce somewhat the errors

that are introduced by the other sources of variance. For instance, if one-third of the variance in milk-yield of a breed of dairy cows is genetic in this narrow sense of the word, and if the breeders pay no attention to bulls, but select the cows so intensively that those saved for breeding average 150 gallons more than their entire contemporary generation, one can expect the next generation to average 25 gallons above the generation in which their parents were born. When compared with the 150-gallon 'selection differential', the 25-gallon gain may appear disappointingly small. No doubt this explains the fact that in every country the voices of zealous enthusiasts are sometimes heard proclaiming that the old methods of selection and attention to pedigree are useless, but that the latest panacea of progeny test, sire index, or what not, will accomplish all that the old methods are condemned for not doing.

In the example just quoted, the complete and exhaustive use of a progeny test for the selection of bulls, while still using the same standard of selection for the cows, could about double the rate of progress, the exact figure depending much upon what portion of the tested sires it is finally necessary to save for replacement purposes, and on what portion of the dams of the future breed are sired by bulls being tested, and what portion can be sired by bulls after they have been proven. Such a doubling of the rate of progress would be an achievement important enough to justify much enthusiasm for proven sires. Yet the point I wish to make here is that the difference is not between methods that are wholly right and methods that are wholly useless, but between methods of selection that use more or use less of the information which is available or might be made available for selecting. The difference is in degree more than in kind. Too often the old method is condemned because it does only what a more careful study and measurement would lead us to expect it to do, whilst a method heralded as new is, on account of its plausibility, extolled far beyond what we really have a right to expect of it.

For further example : if the selection practised were only that of saving bulls from the high-producing cows, what has one a right to expect ? That is tantamount to predicting cows' yields from the yields of their paternal grandams, and one would expect such cows to exceed the average of what their generation would have yielded without selection, by an amount approximately equal to one-fourth of that fraction of the variance which is additively genetic, multiplied by the amount by which the paternal grandams' yield actually exceeded the average of their generation. Thus, with one-third of the variance genetic and a selection differential of 150 gallons for the paternal grandams one would expect an increase of only about 12½ gallons in the grand-daughters. This might naturally seem almost no increase at all to those who had been led by innuendo to think that they had a right to expect in the grand-daughter an increase almost equal to the selection differential practised on the grandams.

These calculations are crude and sketchy and do not include, for example, what may be expected when selection is practised simultaneously on dams and sires, grandams and grandsires, but naturally with varying intensities on each, and with

varying degrees of knowledge about each. These illustrations have been used to show what kinds of knowledge are needed, and how one should proceed to make a business-like estimate of the probable gains to be derived from various methods of selection, progeny-testing, etc. Not until such a valid estimate is made, is one in a position to decide whether a given procedure is likely to provide gains that will more than offset its costs. In preparing these estimates, there is work in plenty to be done in measuring reproductive rates, selection differentials reasonably attainable, the genetic portion of the observed variance, etc.

In addition to the genetic variance that can be expressed by an additive scheme, there is a portion (perhaps a very large portion in stocks that are already improved) of the variance which is genetic in a broad sense, but cannot be expressed additively because the gene combination interact in other ways. Such gene interactions include inhibitory, multiplicative, complementary and epistatic effects, and also what may be a very common class of cases where that which is optimum in the breeder's opinion is genetically intermediate.

No of Plus Genes.
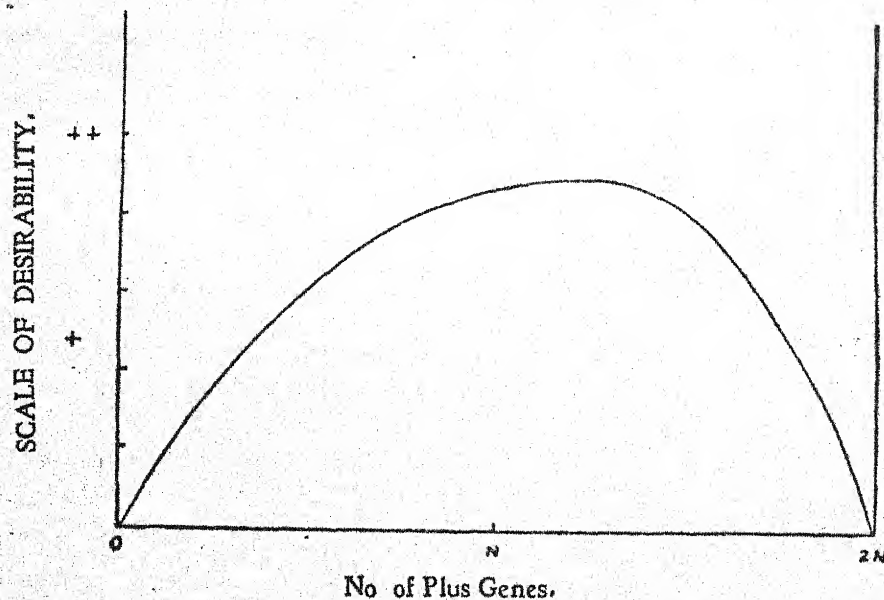
SCALE OF THICKNESS OF BACK FLESH.

Diagram illustrating the case where the maximum of desirability may be produced by an intermediate combination of genes. In such a case selection usually produces noticeable results in the first few generations it is practised, but rather quickly approaches a limit beyond which progress is not made, although hereditary variability still remains. Further progress can often be made if some combination of inbreeding and outbreeding is practised along with continued selection.

For instance, in bacon swine too thick a back is as undesirable as a back too thin. Hence, although the genes affecting thickness of back might perhaps act additively in terms of linear measurements to make the back thicker or thinner, yet they would not act additively when measured on the scale of how much more or less desirable they make the bacon carcass. That is illustrated schematically in the accompanying diagram. Another example, outwardly different but the same in principle, may be furnished by dressing per cent in swine. Other things being equal, the higher the dressing per cent the more desirable the pig from the butcher's view point. From the grower's standpoint there must be some limit to this. The pig must have a certain weight and volume of digestive and other vital organs if it is to be healthy and thrifty. Hence, considering everything, the ideal pig in this respect will be something of a compromise between two partially irreconcilable ideas, having a dressing per cent somewhat lower than the butcher wishes but somewhat higher than the grower would wish if he could afford to disregard the butcher's desires. It is characteristic of such cases that the individual genes cannot be rated as either good or bad, since the directions of their effects are so dependent upon the combination of other genes present. Hence selection is for or against gene combinations as such, rather than for or against the constituent genes of those combinations. On the other hand, the mechanism of inheritance is such that the genes and not the gene combinations are the units of inheritance.

The problem presented by such variance can be solved by the use of inbreeding to differentiate the stock into genetically distinct strains or families, with rare outcrosses between such families to restore the variance and make new combinations possible. Such a differentiation into lines should permit inter-family selection, which would be more effective than individual selection. However, the proper balance to be maintained between the intensity of inbreeding, frequency of outcrossing, and intensity of individual and family selections, in order to promote the maximum rate of progress, offers a whole series of problems, the complete solution of which may well vary from case to case and require continued research for years to come on a scale not yet approached in any country.

In summary, then, we are agreed that inheritance is complex, and that each trait must be studied for itself from several points of view. We are also agreed that the first practical step in breeding is selection under an environment which will permit the genetic differences between individuals to manifest themselves as definitely as possible. We are agreed that progeny tests and, at least, a little initial attention to pedigree are quite helpful. We are not agreed as to the relative amounts of attention that should be given to individual performance, pedigree, and progeny test. We are agreed that it is desirable (as Mr. Smith especially has emphasized) to control environment as much as possible, so as not to be misled by it when making selections, but we differ in our optimism as to how complete control can be in actual practice. We are not entirely agreed as to the gains that

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will accrue from a complete and detailed knowledge of the technical genetic situation in each case. We are far from agreeing on the importance which inbreeding and outcrossing should receive in breeding for productivity.

I hope that such discussion as this, which of itself solves no problems, may nevertheless contribute to their solution by formulating them more clearly and particularly by presenting to our colleagues in more classical branches of biology some view of the nature and complexity of the problems that are still unanswered. Measurement looms large in such problems. This explains the importance of quantitative methods in applied genetics.

ABSTRACTS

Die Stallrotkrankheit des Rindes. (Chronic Haematuria of Cattle)
Schlegel M. *Munch. Tierartz. Wchft.* **85**, 365—371 and 380—384.

Schlegel's first article on haematuria was published in 1912. With the official support of the Ministries of the Interior and of Agriculture of the Reich, a very comprehensive investigation has been carried out since 1927. The results are now recorded in a series of documented articles. With the collaboration of a geologist, a botanist and a chemist, a scientific survey of haematuria localities—the so-called " hunger farms " of the Black Forest in Germany, has been carried out. Details of geological and chemical characters of the soil of haematuria farms, their water supply and botanical flora, the chemical and physiological characters of blood and urine, the clinical and *post mortem* features, associated with the disease have been studied. Affected farms are found to contain granite soil and sandstone, and the hay to be rich in crude fibre but deficient in albumin, mineral salts and vitamins. Water supply contains acids but is poor in mineral salts. The author is inclined to ascribe causation to these deficiencies, and his control measures are evolved from this stand-point. Microscopic and cultural examinations and transmission experiments in large and small animals have been carried out, and on the basis of the negative results obtained, he excludes emphatically, bacterial infections (Detroye, Galtier and Bondeau), animal parasites, coccidia (Arnold, Scharer), Filariae and Distomes (Lydtin), Pentastomes (Burton), passive hyperaemia and round cell sarcoma of the bladder (Hink), toxic and mechanical outflow and unskilled manipulation at the time of birth, and also chemical poisons like Oxalic acid (Hadwen and Roger). The author records that while in one farm all the 22 animals contracted the disease, occasionally sporadic cases were met with.

Commencing with Ross's work of 1878, the author surveys the literature [the previous 40 years' existing literature would appear to be unknown to the author], and designs his transmission experiments to test each of the suggested causes. First to ascertain the truth of the general belief existing for long amongst owners of " red farms " (haematuria) that the disease is somehow transmitted from animal to animal, and to test the bacterial theory, the author collected blood from the jugular vein from clinical cases and administered it whole or defibrinated by either one or more of the intravenous, subcutaneous or oral routes into healthy animals, haematuria urine being given at the same time either orally or subcutaneously. These administrations were repeated several times in each animal, the animals being kept under observation up to 11 weeks until destruction, and their respiration, pulse, temperature and character of the urine noted. 80 c.c. to 200 c.c. of defibrinated or whole blood was given intraven ously or subcutaneously, 50 c.c. to 180 c.c. of urine containing a high percentage of blood was administered subcutaneously and orally, while ¾ litre or more urine was drenched. No abnormality was noted in life or at autopsy, inspite of these large doses being repeated five or more times. Similarly, 65 c.c. of a filtered emulsion of verrucose bladder growths and of the kidney from a clinical case was given subcuta-

neously into a cow, and 5 weeks afterwards when the animal was slaughtered, no changes were discovered at the seat of inoculation or elsewhere. In two of the experimental healthy cows, the calcium content was found to decrease and the phosphoric acid content to increase. Further in order to compensate for the loss of blood in the urine, a quantity of blood from a healthy cow was given intravenously to an affected animal but no amelioration resulted. At autopsy of experimental animals, lesions of tuberculosis have been recorded from most animals but no explanation for this unusual finding has been offered. In the small animal tests, 4 white mice, 3 guinea pigs and 2 rabbits were inoculated with 1, 2 and 5 c.c. of strongly bloody urine at the root of the tail but no reaction was noticed in life or on destruction.

From the above experiments and the failure to find any parasites in the *post mortem* examination of clinically affected cases, the author concludes that the disease is not transmissible, is not contagious or infectious, and no parasite is involved in its causation.

Again to test whether any general poisoning through acids can set up haematuria, commercial acids and spring water from notorious localities were tested experimentally, by administration to healthy animals in such high proportions as neither the fodder nor the water of enzootic areas could possibly contain remembering of course that even if such concentrations were possible in nature, cattle would surely refuse to take them. Increasing doses and varying concentrations of nitric acid (up to 65 c.c. of 0·17 to 0·39 per cent solutions), silicic acid (solution containing up to 50 grms. of Potassium silicate in 0·29 to 1·06 per cent solution) and Oxalic acid (170 grms. of Oxalic acid in 0·2 to 2·43 per cent solution) were given in drinking water over a length of time. No changes were noticed in any of the animals. On the basis of these experiments, the author concludes that these common acids do not cause haematuria in living animals, and that the water from enzootic areas does not contain any virus or bacteria capable of transmitting the disease. [S. C. A. D.].

L'acaprine dans le traitement de la piroplasmose bovine vraie des bovides due a Piroplasma bigeminum (Smith et Kilborns) [Acaprine in the Treatment of true Bovine Piroplasmosis due to Piroplasma bigeminum (SMITH and KILBORNE) Cernaianu, C., Radef, I., & Radescu, T. (1935). *Bull. Soc. Path. Exot.* 28, 804—806.

During 1934, the authors successfully used Acaprine in the treatment of twelve bovines, the majority of which showed very severe symptoms of *Babesia bigemina* infection. The protocols of three of these animals are given and from these it would appear that in all cases a marked amelioration of the clinical symptoms resulted within 24 hours of drug injection. Four other animals, which were in a highly advanced stage of the disease and were lying down on the ground, died within 2 to 4 hours of drug intervention. During 1935, the authors treated fourteen further animals showing inappetence, an icteric condition of the mucous membranes and symptoms of haemoglobinuria, and recovery was obtained in nine of these animals as the result of a single injection of Acaprine.

If the treatment is applied within the first three days of the disease, it usually results in a cure, but in cases of massive infection of the circulating blood, the injection of large doses of Acaprine is liable to cause death as a result of liberation of toxic products from the dead parasites. The drug is also contra-indicated when there are symptoms of acute intestinal disturbance present, such as haemorrhagic enteritis.

The dosage recommended is 2 c.c. of a 5 per cent solution of the drug per 100 kg. (220 lbs.) bodyweight, administered by the intravenous, intramuscular or subcutaneous route. [S. K. S.]

Sur l'avortement epizootique des bovides. Un traitement nouveau. [A new treatment for epizootic abortion of cattle.] R. MOUSSU. (1935), *Rec. Med. Vet.* CXI, 905—919.

The article deals with the elaboration of a new theory to explain the incidence of epizootic abortion and related conditions. Various authors are quoted to support the contention that in the great majority of cases the presence of *Brucella abortus* is not sufficient to induce abortion, and that cows giving a positive agglutination titre and yielding cultures from the milk and blood may yet continue to calve normally.

The theory is developed that *Br. abortus* is an organism which is not primarily pathogenic, but that it is capable of assuming the role of an invader when conditions favourable for its development are created. The fistulous withers of horses that are associated with infection with *Br. abortus* is quoted as an instance. Here the disease is not capable of being artificially reproduced by the injection of virulent cultures of *Br. abortus* deep into the tissues at the point of the withers, but the subcutaneous injection anywhere into an infected horse, of a killed culture of *Br. abortus*, results in the formation of a suppurating local tumefaction, and the presence of living *Br. abortus* in the pus can only be explained as due to migration of the bacilli from the previously existing focus of infection to the site of diminished resistance. The author considers that *Br. abortus* is ubiquitous in nature and that it easily gains an entrance to the blood stream. Circulating with the blood it settles and develops if a site favourable for its development is met with. In the case of fistulous withers, a local injury to the tissues is the predisposing factor. The hygromas of cattle is another instance of the same type.

Epizootic abortion of cattle is considered to be another disease of the same order, except that the primary cause is a different one, being a deficiency in vitamin-E. It is a disease of the foetus and the intensity of the damage caused, is in direct proportion to the degree of deficiency, and is evinced either as sterility, abortions or death of the new-born and retention of the placenta according as the deficiency is nearly total, moderate or only slight. A total deficiency would lead to early death and absorption of the foetus with consequent sterility : and this sequence of events following total deficiency in vitamin-E has already been experimentally proved, by Evans, to take place in the rat.

A partial deficiency in vitamin-E cannot by itself bring about the death of the foetus and it is here that the pathology of the *Br. abortus* infection comes into play. The requirement in vitamin-E of the foetus increases with the increase in its own rate

of growth and in the bovine species it is just between the 5th and 7th month of gestation that the growth rate attains its maximum. At this period, in those cases of moderate deficiency, the supply of vitamin-E falls far short of the requirements of the foetus. Alterations are produced in the foetal tissue which permit *Br. abortus* to localise and cause lesions that lead to abortion.

When the deficiency in vitamin-E is still less, abortion does no longer take place, but the new-born calves soon become victims of pyosepticaemia. The explanation for this lies in the fact that the lesions caused by *Br. abortus*, although not extensive enough to cause abortion, are nevertheless, sufficient to break the placental barriers permitting the infection of the foetus with coli and paracoli of maternal origin. This infection breaks out into a septicaemia a little time after birth when the calf is no longer defended by the antibodies of the mother.

The retention of the placenta is considered to be due to a slight deficiency in vitamin-E which does not permit the invader to cause anything more than a slow inflammatory process and this is said to bring about adhesions with the cotyledons.

The factor E is a fat soluble vitamin, the chemical composition of which is still undetermined. It exists in the sprouts of the graminaceous seeds. From the seeds it passes into the young plant and when these plants come to bear seeds, no trace of the vitamin is left in them. Soil, moisture and atmospheric factors may influence the formation of vitamin-E in the plant. This vitamin is thermostable but is rapidly destroyed by oxidation. The best form of artificially administering it is as an oil expressed from the sprouts of graminaceous seeds.

Animals usually refuse to consume the hard seeds and the young plant is, therefore, the only source of vitamin-E available for them. Incidentally it is mentioned that abortions are not noticed during the spring and the summer when animals get plenty of green fodder. Vitamin-E is excreted through the milk, so the need for this factor is much more in animals that are intensely bred for the yield of milk.

It is claimed that abortion is amenable to treatment based on these assumptions. The treatment consists of three subcutaneous injections of 30 to 40 c.c. each of wheat-germ oil, the first to be injected at the time of service, the second at the end of three months and the third at the end of six. The dosage may require to be increased in regions where the natural deficiency is great. When the dosage is correctly estimated success is complete.

It is also emphasised that the treatment is one of prevention and not one of cure. For the treatment to be effective the first injection ought to be given at the time of service.

The author treated 7,000 cows on the basis of the principles already enumerated, and the claim is made that in all those cases in which proper precautions had been taken as regards dosage and time of intervention, the treatment, whether for abortion, retention or mortality of the new-born was invariably attended with successful results. [V. R. R.]

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A Handbook of Statistics for use in Plant Breeding and Agricultural Problems by Dr. F. J. F. Shaw, C.I.E., D.Sc., A.R.C.S., F.L.S., I.A.S. (*In the Press.*)

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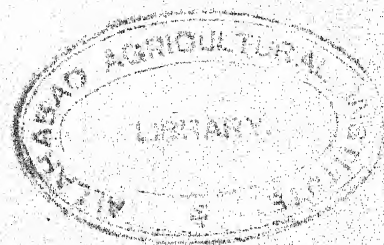
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(Vol. VI, Part IV)

December, 1936

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ORIGINAL ARTICLES

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STUDIES ON THE DIPHTHERITIC FORM OF FOWL-POX IN INDIA

BY

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(With Plates XX & XXI)

INTRODUCTION

Fowl-pox is a contagious disease of birds due to a filtrable virus and is characterised by the appearance of wart-like nodules on the head and cheesy diphtheritic membranes in the buccal cavity with or without oculo-nasal discharge. One or all of the above lesions may be present in the same bird.

At the instigation of this Institute in 1934, specimens in the form of dried crusts from suspected cases of fowl-pox, have been received from various parts of India for confirmation, and this has enabled the writers to study the disease.

As detailed in Table I, so far 22 specimens have been received, out of which 15 were found positive to fowl-pox, and of these, three received from the Madras Presidency gave rise to the development of diphtheritic lesions in the buccal cavity in addition to the cutaneous lesions, on subinoculation over the feather follicles of healthy fowls. Advantage was taken of this opportunity to study this diphtheritic condition in some detail.

A review of the past literature shows that the nomenclature of this disease has been rather confusing. Each lesion appears to have been named separately and amongst other names we have chicken-pox, contagious epithelioma, cp   r, avian diphtheria, and roup, although it is now known that these conditions are only different manifestations of the same disease determined by one and the same virus.

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While studying the diphtheritic form Moore [1885], Marshall [1900] and Jackley [1918] were of opinion that bacteria might be the cause but could not confirm their identity. Jowett [1909] stated that comb and mouth lesions were etiologically distinct. Ward [1904] was the first to relate roup etiologically to chicken-pox. Haring and Kofoid [1911] demonstrated cell inclusions of chicken-pox in the cheese-like lesions of the mouth and throat of fowls. Hadley and Beach [1913] believed that the head and mouth forms were different manifestations of the same disease. Mack and Records [1915] showed evidence that chicken-pox and avian diphtheria were etiologically identical but did not draw positive conclusions. Arloing [1911-12] differentiated false and true fowl-pox diphtheria by histological methods. Jackley [*loc. cit.*] while studying roup, incriminated an organism of the *Pasteurella* group. Beach, Lothe and Halpin [1915] showed in an outbreak of chicken-pox and avian diphtheria that the high mortality was due to secondary invading bacteria although the primary cause was a filtrable virus. From the study of Brumley and Snook [1916] it seems that although there is a virus and complicating conditions induced by secondary bacterial infection, neither factor alone would cause a typical disease. Doyle and Minett [1927] studied the identity of wart-like growths on the head and diphtheritic lesions in the mouth and confirmed that comb and mouth lesions in the case of fowl-pox were due to the same virus and immunity to one form could be set up with material from another form. There was no evidence that bacteria alone could produce typical mouth lesions, the primary action of the specific virus being essential.

PURPOSE OF EXPERIMENTS

Experiments have been conducted to study the transmissibility of the virus of the diphtheritic lesions, its tendency for generalization, its etiological identity with the ordinary cutaneous form of fowl-pox as commonly found in India, filtrability of the causative virus and its immunological identity with our stock fowl-pox virus, which was obtained through the courtesy of the Veterinary Laboratory of the Ministry of Agriculture, Weybridge, England.

METHOD OF EXPERIMENTAL TRANSMISSION

A specimen of crusts was powdered and a 1 per cent emulsion was made after the method of Doyle and Minett [*loc. cit.*] with a slight modification that instead of ordinary saline, 80 per cent glycerinated saline was used in its preparation. The inoculum thus prepared was applied by means of a swab over a scarified portion of the comb in the case of cocks, and in other birds over the feather follicles of the leg after pulling out a few feathers. Two healthy fowls and a fowl immune to our stock fowl-pox virus (Weybridge strain) were used for each specimen and the results were reported after a fortnight's observation from the

date of inoculation, except in the case of negative results, when the fowls were subjected to an immunity test with the stock virus to exclude the possibility of their having a natural immunity to fowl-pox.

In no case, except for the special purposes recorded in this paper, was direct inoculation ever done over the broken or unbroken mucous membrane of the mouth.

All the specimens which were received from outside were subjected to biological test, for evidence of fowl-pox infection, in the manner described above, with the following results :—

TABLE I

Results of biological test on specimens, for evidence of fowl-pox infection

Serial No.	Speci-men No.	Received from	Date of receipt	Nature of material	Result of biological test		Remarks.
					Healthy fowls	Immune fowls (Wey-bridge strain)	
1	56	Principal, Madras Veteri-nary College.	9th Feb., 1935 .	Dried crusts.	Positive	Negative	Cutaneous le-sions.
2	69	Superintendent, Civil Veterinary Department, Bangalore.	14th Feb., 1935	Do. .	Do. .	Do. .	Do.
3	70	Veterinary Assistant Surgeon, Jamtara, (B. & O.).	15th Feb., 1935	Do. .	Do. .	Do. .	Do.
4	85	Special Officer, C. V. D., Muzaffarpur, (B. & O.).	22nd Feb., 1935	Do. .	Do. .	Do. .	Do.
5	117	V. A. S., Conjeevaram, (Madras).	14th Mar., 1935	Do. .	Negative	Do. .	...
6	121	D. V. S., Nagpur, (C. P.) .	15th Mar., 1935	Do. .	Positive	Do.	Cutaneous le-sions.
7	122	V. A. S., Cuddalore, (Madras).	Do. .	Do. .	Negative	Do. .	...
8	123	Principal, Madras Veteri-nary College.	Do. .	Do. .	Do. .	Do. .	...
9	130	V. A. S., Kumbakonam, (Madras).	18th Mar., 1935	Do. .	Do. .	Do. .	...
10	139	D. V. S., Lucknow, (U. P.).	22nd Mar., 1935	Do. .	Unfit for examination		?
11	150	V. A. S., Rajapalyam, (Madras).	25th Mar., 1935	Do. .	Positive	Negative	Cutaneous le-sions.
12	151	V. A. S., Kothapeta, (Madras).	Do. .	Do. .	Do. .	Do. .	Do.
13	197	V. A. S., Anakapalle, (Madras).	20th April, 1935	Do. .	Do. .	Do. .	Do.
14	220	V. A. S., Deoria, (U. P.). .	4th May, 1935 .	Do. .	Negative	Do. .	...
15	246	V. A. S., Kumbakonam, (Madras).	16th May, 1935	Do. .	Do. .	Do. .	...

TABLE I—(continued)

Serial No.	Specimen No.	Received from		Date of receipt	Nature of material	Result of biological test		Remarks.
						Healthy fowls	Immune fowls (Weybridge strain)	
16	285	V. A. S., (U. P.).	Hassanpur,	12th June, 1935	D r i e d crusts.	Positive	Negative	Cutaneous lesions.
17	397	V. A. S., (Madras).	Cannanore,	20th Aug., 1935	Do. .	Do. .	Do. .	Cutaneous and buccal lesions.
18	429	Ditto	.	13th Sept., 1935	Do. .	Do. .	Do. .	Do.
19	39	Ditto	.	20th Jan., 1936	Do. .	Do. .	Do. .	Cutaneous lesions.
20	84	V. I. O., (U. P.).	Lucknow,	15th Feb., 1936	Do. .	Do. .	Do. .	Do.
21	129	V. A. S., (Madras).	Chinglepnt,	11th Mar., 1936	Do. .	Do. .	Do. .	Do.
22	193	Ditto	.	6th April, 1936	Do. .	Do. .	Do. .	Cutaneous and buccal lesions.

Conclusion :—From the above table, it is evident that the fowls immunized with the Weybridge strain of fowl-pox virus can resist the Indian strain of this virus, proving their immunological identity.

TRANSMISSION EXPERIMENTS

On subinoculation over the feather follicles of the leg, emulsion prepared from specimen No. 397/1935, received from Cannanore, Madras, gave rise to the development of fowl-pox lesions over the leg and diphtheritic membranes in the buccal cavity in the case of healthy fowls which eventually died, whereas the immune bird showed no lesions of the disease and remained quite healthy. *Post mortem* examination on the dead fowls revealed extensive buccal lesions extending into the throat (Plate XX, Fig. 1). On histo-pathological examination, the usual features of a diphtheritic lesion were observed and on cultural examination *Escherichia coli communior* and *Streptococcus faecalis* were isolated.

Death was not very common in fowls exhibiting cutaneous lesions only, and in the above case the fowls evidently died on account of the acute buccal form of the disease.
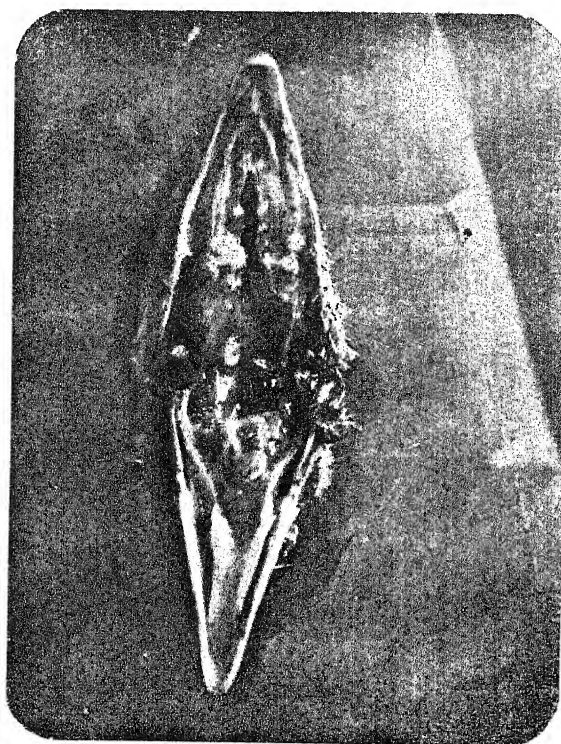
FIG. 1.   Buccal diphtheritic lesions of fowl No. 229.
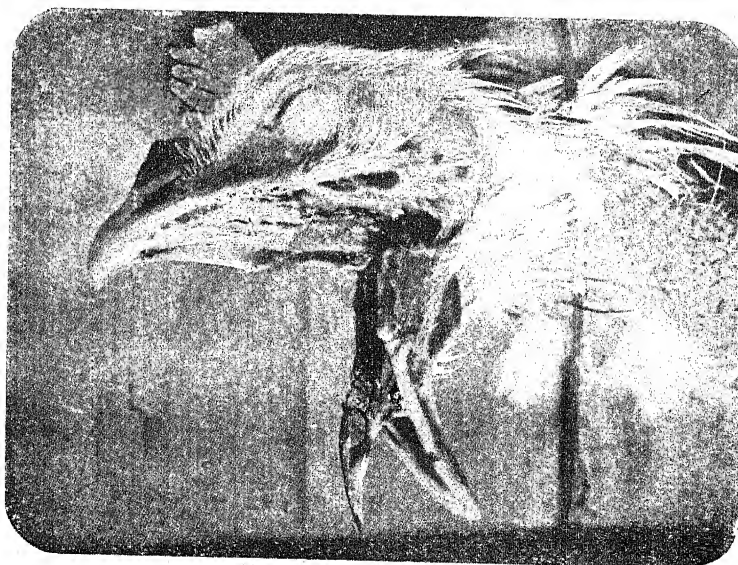
FIG. 2.   Buccal diphtheritic lesions reproduced in fowl
No. 256, from the buccal material of fowl No. 229.

Two more specimens (Nos. 429/1935 and 193/1936) were received which on biological test also gave rise to the development of the buccal form of the disease in addition to localisation over the feather follicles of the inoculated leg in healthy fowls, whereas the immune fowls did not react.

In neither of the above cases was the virus emulsion applied in the mouth but on the leg only.   From the above tests it is confirmed that the etiology of the mouth form and the cutaneous form is one and the same, and the virus in the above cases has produced the mouth form without any direct inoculation into the buccal mucous membrane.   In other words it seems that the above samples of crusts on subinoculation into healthy fowls induced a reaction of a greater severity and probably caused generalisation of the disease.

TABLE II

Transmission experiments

Specimen No. 397/1935

One per cent emulsion of crusts in 50 per cent glycerinated saline inoculated on 2nd September, 1935 over the feather follicles.

Healthy

Immune to fowl-pox virus (Weybridge strain).   Fowl No. 218 (no reaction).

Fowl No. 229 (reacted, leg and mouth lesions ; died on 25th September, 1935).

Fowl No. 228 (reacted, leg and mouth lesions ; died on 26th September, 1935).

Saline emulsion of the diphtheritic material obtained from the buccal cavity, inoculated into two healthy fowls on 27th September, 1935.

Over the feather follicles.
Fowl No. 255 (reacted, leg and mouth lesions ; died on 28th October, 1935).

Over the scarified buccal mucous membrane.
Fowl No. 256 (reacted, buccal lesions reproduced ;   destroyed   on 22nd   October, 1935 for photographic purposes.)

Glycerinated saline emulsion of the crusts obtained from leg lesions, inoculated over the scarified buccal mucous membrane of two healthy fowls on 15th November, 1935, and about a dozen feathers pulled out from a leg in each case.

Fowl No. 303 (reacted, typical buccal lesions only ; died on 24th November, 1935).

Fowl No. 304 (reacted, typical buccal lesions only ; died on 23rd Nov., 1935).

The virus is being maintained by passage through fowls.

From Table II it will be observed that inoculation of the leg alone in the case of fowl No. 255 gave rise to the lesions in the mouth in addition to the localisation over the feather follicles of the leg, giving a suggestion of generalisation of the virus in the body of the fowl (Plate XXI). Fowl No. 256 which was inoculated over the scarified buccal mucous membrane showed the development of lesions only in the mouth and not in any other part of the body where all the feathers were intact (Plate XX, Fig. 2). This is in accord with the findings of Doyle and Minett [loc. cit.] who failed to infect a fowl's leg from which feathers had not been plucked.

Two fowls Nos. 303 and 304 were subinoculated with the virus in the mouth on 15th November 1935 (Table II), and at the same time feathers were plucked from the leg but no application of the virus was done on that site in either case. Both died after a week showing severe diphtheritic lesions in the buccal cavity, but without showing any lesion over the plucked area. The severe mouth lesions apparently caused the death of the birds too quickly to allow the cutaneous lesions to develop through generalisation.

Two fowls were scarified in the mouth with a sterile scalpel but no virus was inoculated. No lesions were observed in the mouth in either case, indicating that the micro-organisms normally inhabiting the buccal cavity of fowls were not able to produce the diphtheritic lesions without the presence of fowl-pox virus.

VIABILITY OF THE VIRUS IN FILTRATES

Material for filtration was obtained from the buccal lesions of two fowls and cutaneous lesions of fowl No. 255, in which the lesions were produced from the same original diphtheritic buccal material of fowl No. 228. The material was gently removed with sterile forceps, and, after a mild wash in normal saline solution, was pulped and emulsified with the same solution to make a 1 per cent emulsion. The emulsion was left at room temperature before filtration for twenty minutes for autolysis to take place. Filtrates were obtained through Chamberland L. 3, Berkefeld 'V' and Berkefeld 'N'. Every precaution was taken to avoid leakage and contamination during the process of filtration. Healthy fowls were inoculated with the filtrates, as detailed below in Table III and were subsequently put to immunity test with the stock virus, with the result that Berkefeld 'V' filtrate was found to be viable whereas Chamberland L. 3 and Berkefeld 'N' filtrates were found to be sterile.

Ind. Jour. Vety. Sci. & Anim. Husb., Vol. VI, Pt. IV.

PLATE XXI
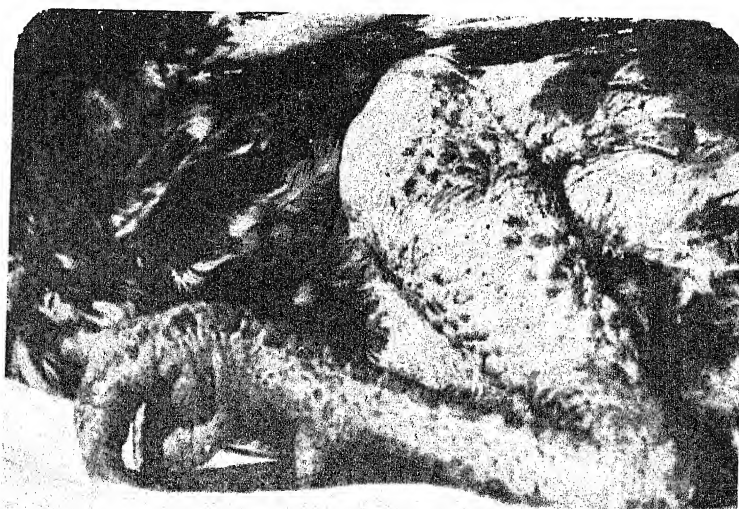
Leg lesions produced in fowl No 255, from the buccal material of fowl No. 229. (This fowl also showed diphtheritic lesions in the buccal cavity.)

TABLE III

Filtration Experiments

Serial No.	Fowl No.	Date of inoculation	Site of inoculation	Inoculum*	Reaction	Result of immunity test with fowl-pox virus (Weybridge strain)	Remarks.
1	282	22nd Oct., 1935	Feather follicles and buccal mucous membrane.	Chamberland L. 3 filtrate (Buccal material from fowl No. 256).	Negative .	Reacted .	Aviable.
2	305	19th Oct., 1935	Feather follicles	Berkefeld 'V' filtrate (Cutaneous material from fowl No. 255).	Died on 28th Nov., 1935 due to other causes.	...	?
3	306	Do.	Do.	Do.	Positive leg and mouth lesions.	No reaction.	Viable.
4	218	2nd Dec., 1935.	Do.	Berkefeld 'N' filtrate (Buccal material from fowl No. 297).	Negative .	Destroyed on 17th Dec., 1935 for experimental purposes.	Aviable.
5	247	Do.	Do.	Do.	Do. .	Reacted .	Do.

* The material in all the five cases was derived from a single strain (Buccal lesions of Fowl No. 228 which reacted to specimen No. 397/1935).

DISCUSSION

From the results of the transmission experiments it is evident that the diphtheritic form of fowl-pox is also observed in India, besides the cutaneous form, and that the two forms are due to the same virus, which is immunologically indistinguishable from our stock Weybridge strain of this virus.

Positive results with the Berkefeld 'V' filtrate of the material originally obtained from the buccal cavity of fowl No. 228, exhibiting the diphtheritic form of fowl-pox and inability of the micro-organisms normally inhabiting the buccal cavity of fowls to produce such lesions without the help of the virus, in spite of scarification, show that the above described lesions are due to a specific virus.

With this particular strain of virus, buccal lesions developed almost invariably, in addition to the cutaneous lesions, even when the material was inoculated only on the feather follicles. But when the buccal cavity was inoculated with the material it killed the bird rather quickly and no distant cutaneous lesions were able to develop through generalisation, in spite of pulling out the feathers and thereby injuring the feather follicles. It was only in one case (fowl No. 256)

that the bird lived for about four weeks after the inoculation of the material in the buccal cavity, possibly due to varying individual susceptibility, but in that case the feathers had not been plucked and lesions were noticed only in the mouth. The explanation appears to be that unbroken skin does not take the virus from the body. Lesions of the oculo-nasal form of the disease were also observed in subsequent passages of the same virus.

Doyle and Minett [*loc. cit.*] described the possibility of generalised symptoms being set up by inoculation of the virus intravenously, subcutaneously and intra-muscularly, although the lesions developed in a varying degree of severity.

In comparison with those cases where only localised cutaneous lesions developed, there was a greater tendency for fatal termination when generalisation occurred, with the additional production of mouth lesions as was observed with the strain under study. In view of its highly virulent nature it is being maintained at this Institute.

SUMMARY

(1) A survey of the past work on fowl-pox and several other allied conditions, now known to be etiologically one and the same, has been made.

(2) Experimental transmission of diphtheritic lesions from the mouth of fowls has confirmed that the causal agent, a filtrable virus, is the same as that which produces fowl-pox lesions on the skin in India.

(3) The Indian strain of fowl-pox virus is immunologically indistinguishable from the Weybridge strain.

(4) There seems to be a tendency for generalisation with this particular strain of the virus (obtained from Cannanore, Madras) although this has not been seen in the case of specimens received from other provinces in India, so far.

(5) Filtration experiments have been conducted with this strain of the virus and Berkefeld 'V' filtrate has given positive results unlike Chamberland L. 3 and Berkefeld 'N' filtrates.

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O

OBSERVATIONS ON DOYLE'S DISEASE OF FOWLS

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Doyle's disease, which is commonly known in India as Ranikhet disease, is a disease of the greatest economic importance as it ranks first amongst the diseases of fowls in causing mortality. It was first recognised in the United Kingdom by Doyle in 1926 and in India by Dr. Edwards in 1927. Since then it has been found prevalent in certain other parts of the world ; Piecard found it in Java in 1928, Rodier in the Philippine in 1929 and Ochi and Hashimoto in Korea in the same year. In the Bombay Presidency the disease was first recognised by me in the year 1932 and materials from the artificially infected fowls were forwarded to the Imperial Institute of Veterinary Research, Muktesar, for confirmation. It is also understood that the disease is very extensively prevalent in various parts of India. It is a fatal disease and whenever it breaks out in a village it wipes out the major portion of the fowl population if not the whole and as a result poultry-breeding industry is sustaining very heavy losses every year.

Unfortunately no curative treatment or prophylaxis against the disease has been found out as yet. All efforts made by different workers in this respect have, so far, proved abortive ; Doyle [1927] tried heat-killed virus without success ; he, however, experimented with an anti-serum prepared from ducks and found that birds treated with it failed to contract the disease by contact but died when artificially infected. Cooper [1931], on the other hand, succeeded in protecting birds against artificial infection by injecting serum prepared from immune fowls. But as the quantity of serum obtained from a fowl or a duck is extremely small this contribution to the knowledge of immunology has served no practical purposes in dealing with the disease. Doyle [1927] further found, in Great Britain, that formalinised virus when applied by the subcutaneous route afforded considerable protection against natural infection but not against artificial one, whereas all attempts made in India at the Imperial Institute of Veterinary Research, Muktesar, to prepare such a product either by treating the vital organs with chloroform or formalin or by desiccating them *in vacuo* have proved of no avail.

In the lay press in India one frequently comes across reports, evidently written by fowl-breeders having little knowledge of the disease, and medicines claiming good results obtained by the oral administration of kerosine oil or some other stuff. But it is needless to say here that they have proved utterly useless in saving the life of affected fowls.

In the Western countries the disease is stamped out soon after its appearance by destroying the affected and in-contacts and by thorough disinfection under legislation. It is possible to do this there easily without great loss as the disease is of rare occurrence. But, in India, the disease has found a permanent home and it is impossible to control it as there is no such legislation. Even if the latter be enacted, still it would not be possible to control it on account of its extensive prevalence all over India and the paucity of the members of the Civil Veterinary Department in the Provinces. The only course left to India, is, therefore, to find out an efficient prophylaxis against the disease.

In the past I tried intravenous injections of formalin and Lugol's solutions in a certain number of outbreaks without any success. I have also tried the oral administration of the concentrated solution of potassium permanganate in brandy and 0·3 per cent hydrochloric acid in milk at the recommendation of some American Missionary people in India with the same negative results. During the last Christmas the disease was found prevalent at the Ramabai Mukti Mission compound and I tried intravenous and subcutaneous injections of Trypanblue at the doses of 1 to 4 c.c. of 1 per cent solution as a curative and preventive treatment and found that the drug possessed considerable prophylactic value. Systematic experiments were thereupon undertaken to ascertain the extent of this value under artificial and field conditions.

Prophylactic value of Trypanblue under artificial conditions

Experiment 1.—Twenty birds which were susceptible to the disease were subjected to the experiment as follows :—

    3 fowls injected with Trypanblue and infected artificially on the 7th day.

    3 fowls injected with Trypanblue and infected artificially 24 hours after.

    2 fowls injected with Trypanblue and infected artificially simultaneously.

    3 fowls injected with Trypanblue and exposed to contact infection on the 7th day.

    3 fowls injected with Trypanblue and exposed to contact infection 24 hours after.

    2 fowls injected with Trypanblue and exposed to contact infection simultaneously.

    2 control fowls infected artificially.

    2 control fowls exposed to contact infection.

    ——
    20
    ——

The dose of Trypanblue was 2 to 3 c.c. of 1 per cent solution in normal saline. The birds which were simultaneously injected with the virus or exposed to infection received the drug intravenously and the others by the subcutaneous route. All birds were kept in a small room and were forced to eat and drink from common feeding and drinking troughs respectively. Sick birds were not

fed by hand. The temperature of the birds was recorded twice daily. Details of these birds are given in Table I. The following is the abstract of the same :—

Serial No.	Fowl	Interval between the injection of trypanblue and infection	Date of injecting		Date of exposing to contact infection	Date of death	Interval between the date of exposure to infection and death
			Trypanblue	Virus			
1	Cock	One week.	19th Jan., 1936.	26th Jan., 1936.		6th Feb., 1936.	11 days.
2	Hen		Do.	Do.		30th Jan., 1936.	4 "
3	Hen		Do.	Do.		31st Jan., 1936.	5 "
4	Hen		Do.	Nil	26th Jan., 1936.	2nd Feb., 1936.	7 "
5	Hen		Do.	Do.	Do.	7th Feb., 1936.	Ill and was destroyed.
6	Hen		Do.	Do.	Do.	4th Feb., 1936.	9 days.
7	Hen	24 hours.	25th Jan., 1936.	26th Jan., 1936.		29th Jan., 1936.	3 "
8	Cock		Do.	Do.		30th Jan., 1936.	4 "
9	Hen		Do.	Do.		31st Jan., 1936.	5 "
10	Hen		Do.	Nil	26th Jan., 1936.	2nd Feb., 1936.	7 "
11	Cock		Do.	Do.	Do.	4th Feb., 1936.	9 "
12	Cock		Do.	Do.	Do.	4th Feb., 1936.	9 "
13	Cock	Simultaneously.	26th Jan., 1936.	26th Jan., 1936.		29th Jan., 1936.	3 "
14	Cock		Do.	Do.		30th Jan., 1936.	4 "
15	Cockrel		27th Jan., 1936.	Nil	27th Jan., 1936.	2nd Feb., 1936.	7 "
16	Cockrel		Do.	Do.	Do.	7th Feb., 1936.	Ill and was destroyed.
17	Pullet	Controls	Nil	26th Jan., 1936.		31st Jan., 1936.	5 days.
18	Pullet		Do.	Do.		29th Jan., 1936.	3 "
19	Pullet		Do.		26th Jan., 1936.	2nd Feb., 1936.	7 "
20	Pullet		Do.		Do.	31st Jan., 1936.	5 "

All fowls got affected with the disease and died within twelve days except two which too were ill and were, therefore, destroyed to wind up the experiment in order to enable me to attend to urgent duties awaiting elsewhere. These results show that trypanblue failed to protect the birds when they were infected artificially or exposed to heavy infection under artificial conditions.

Prophylactic value of Trypanblue under field conditions

Experiment 2.—This experiment was carried out at Kedgaon, a village in Poona District. The disease had broken out there on 20th December 1935 and had inflicted a heavy loss to the village as more than 300 fowls had died. The experiment was undertaken on the fowls which were located in one street where the statistics were 88 attacks, 87 deaths and one " still affected ". Two hundred

and fourteen fowls were protected with trypanblue solution at the rate of 1 to 3 c.c. according to age. The drug was injected intravenously in some and sub-cutaneously in others. All fowls were allowed to move about as usual and re-main in their own pens. Only one of these birds showed symptoms of the disease on the 7th day after injection, which was thereupon destroyed and burnt. All other birds remained perfectly healthy. Details of these fowls are given in Table II.

The results obtained in this experiment show that trypanblue afforded ade-quate protection to fowls subjected to its injection and maintained under field conditions.

Experiment 3.—A similar experiment as the one preceding was undertaken at the village Bori, in Poona District. The disease had broken out there on 7th January 1936 and there were 123 attacks, 115 deaths and 8 " still affected ". Fifty fowls which were apparently healthy were protected with trypanblue at the doses mentioned already, in the affected locality and were allowed to remain under natural conditions. Affected fowls were neither isolated nor destroyed although owners were advised to do so. Amongst the fowls protected, three died of the disease : one on the third day and two within a course of a week. All others failed to contract the disease while, it is reported, that about 50 un-protected fowls died subsequent to the prophylactic injections carried out in the village. Details of the protected fowls are given in Table II.

These results show that 94 per cent of the fowls protected with trypanblue failed to contract the infection of the disease under field conditions.

Experiment 4.—While I was carrying out experiment 1 at Kedgaon, Rev. J. E. Norton, B. C. H. Mission, Dhond, personally requested me to save the fowls of the Mission workers at Dhond from the jaws of this disease which was pre-valent there since 14th January 1936. Two hundred and twenty-four fowls had already died of the disease and there were six affected. Reserve Veterinary Assistant Surgeon, Nemade, who was attached to my office temporarily was de-puted there, who injected 142 fowls which were apparently healthy with the same dose of trypanblue as stated in the previous pages. They were allowed to move about as usual and to remain under the same field conditions as they were before. I checked the results 51 days after and found that the disease had subsided about 9 days after the injection and that 29 fowls from the protected lot had died of the disease ; 13 died within 5 days, 15 showed dullness from the second day after injection and died within a course of a week and 1 died on the 8th day. Details of these birds are given in Table II.

These results show that the percentage mortality among the birds protected is 20·4. But, it would appear, by a close study of these fowls and referring to Table II that out of the 29 fowls 28 were in the incubation stage of the disease at the time of injection. For, in experiment 1, it has been seen that the minimum

period during which death has occurred as a result of contact infection is five days. If all deaths that have occurred within this period and also those which have occurred within a week following dullness from the second day are excluded the mortality figure comes to only one, thus giving a percentage mortality of 0·7.

It is a known thing that whenever the disease appears in a fowl house or in a closely inhabited locality as that of the Mission compound, Dhond, all fowls of the house or locality succumb to it save a small percentage up to 5. But the fact that more than 80 per cent of the fowls failed to contract the disease at this institution goes to show that trypanblue had exerted a protective action against it.

Summary of experiments 2, 3 and 4

Experiment No.	Village	Fowls injected with Try-panblue	Total deaths	Deaths excluding those having pre-injection infection
2	Kedgaon	214	1	1
3	Bori	50	3	2
4	Dhond	142	29	1
	Total	406	33	4
	Percentage mortality	..	8·1	1·0

These results indicate that trypanblue surely possesses a prophylactic action against Doyle's disease provided the fowls protected with the drug are maintained under field conditions where the concentration of the infective material does not occur to any great exent due to the high destructive action of the heat, light and air. The phenomenon of the protection afforded by trypanblue is believed to be due to the saturation of the body cells with the drug, thus producing an unfavourable condition for the minimal doses of the virus ordinarily picked up under field conditions to multiply in the body.

CONCLUSION

Under the existing knowledge of the disease and from the results reported here, it would appear, that the best plan to control the disease is to destroy the affected and to divide the in-contact fowls into groups of 1 to 5. All healthy and in-contact fowls should be injected with trypanblue at the rate of 1 to 3 c.c. of 1 per cent solution subcutaneously or intravenously, the latter being preferable. All fowls should be allowed to run about in the sun or in the open. Potassium permanganate which has the highest virucidal action over the virus of this particular disease according to Doyle [1927] should be mixed in the

drinking water which should be changed every two to three hours during the prevalence of the disease. Examination of the birds should be carried out daily and those showing symptoms of illness should be destroyed in order to remove the infection forthwith.

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TABLE II—contd.

Serial No.	Owner's name	Locality	Date on which disease broke out	Number of fowls died previous to injection	Number still affected	Date of injection of trypan-blue	Number of fowls injected with trypan-blue	Deaths among the injected fowls	Remarks
	Bori.								
1	Ganpati Dashrath Deshmukh.	Bori, Dist. Poona.	7th Jan., 1936.	..	..	23rd Jan., 1936.	8	..	One died on the 3rd day.
2	Maruti Govind Parit.	Do.	Do.	12	..	Do.	2	1	
3	Narayan Govind Deshmukh.	Do.	Do.	..	..	Do.	3	..	
4	Tukaram Ankushrao Deshmukh.	Do.	Do.	..	..	Do.	1	..	
5	Mahadev Tatyaba Deshmukh.	Do.	Do.	..	..	Do.	7	..	
6	Satva Sukhya Deshmukh.	Do.	Do.	39	1	Do.	1	..	
7	Bandubhai Yesubhai Mulva.	Do.	Do.	4	..	Do.	1	..	
8	Savitribai Narayan Tadge.	Do.	Do.	..	..	Do.	27	2	Two hens injected with trypanblue and four not injected died of the disease within about a week.
9	Dashrath Govind Ramoshi.	Do.	Do.	11	3	Do.	..	..	
		Total	.	66	4	..	50	3	
		Percentage mortality	.	..	..	..		6·0	

	Rev. J. E. Norton.	B.C.H. Mission Compound, Dhond, Dist. Poona.	14th Jan., 1936.	22		29th Jan., 1936.	16	2	
Dhond									
1	Rev. J. E. Norton.	B.C.H. Mission Compound, Dhond, Dist. Poona.	14th Jan., 1936.	..	..	29th Jan., 1936.	16	2	Two fowls died three days after.
2	P. D. Parmar	Do.	Do.	..	..	Do.	33	..	Eleven birds got affected with the disease out of which ten including the 2 affected died as follows:— Died. 30th Jan., 1936 . 2 31st Jan, 1936 . 4 1st Feb., 1936. . 1 2nd Feb., 1936. . 2 3rd Feb, 1936 . 1
3	B. V. Hivale	Do.	Do.	14	2	Do.	15	10	
4	Shantabai M.	Do.	Do.	23	..	Do.	6	..	
5	P. B. Sonaware	Do.	Do.	13	1	Do.	1	..	
6	Nathibai M.	Do.	Do.	8	..	Do.	7	1*	
7	Rupaji	Do.	Do.	Sold as they were affected.	..	Do.	1	1†	One bird recovered but is unable to walk easily due to paralysis of the right leg. * One died after 8 days. † Died on the third day. ‡ Affected at the time of injection. It suffered and recovered but has developed incordination of head and neck.
8	Honaji Hotaji	Do.	Do.	3 Affected sold.	1	Do.	1	..	
9	Shivibai	Do.	Do.	29	1	Do.	1	..	
10	Nathuji	Do.	Do.	4	1	Do.	1‡	..	
11	Mohanrao	Do.	Do.	30	1	Do.	2	..	
12	Laxmanrao	Do.	Do.	18	..	Do.	2	..	
13	Ratanbai	Do.	Do.	4	..	Do.	1	..	
14	Hakka Gaman	Do.	Do.	12	..	Do.	3	..	
15	Bahira Master	Do.	Do.	10	..	Do.	2	..	
16	Dayabai Purandare	Do.	Do.	1	..	Do.	2	..	
17	Royalbai N.	Do.	Do.	2	1	Do.	1	..	
18	William K.	Do.	Do.	4	..	Do.	1	..	
19	Ladiabai O.	Do.	Do.	12	..	Do.	2	..	
20	Chaturbai	Do.	Do.	15	..	Do.	2	..	
21	Silas Somaji	Do.	Do.	..	..	Do.	15	15	All showed dullness on the next day and died within a week.
22	Yadu Genu	Do.	Do.	..	..	Do.	27	..	
		Total .		224	6	..	142	29	
		Percentage mortality		..	..	..	..	20·4	

OBSERVATIONS ON THE TREATMENT OF BOVINE NASAL SCHISTOSOMIASIS

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Introduction

Bovine nasal granuloma, or to be more accurate bovine nasal schistosomiasis, seems to exist only in India so far as is known at present. Recently it has been reported from Ceylon. There are no records available to show how long ago this disease made its appearance in India, but Malkani [1933] says that its prevalence has been known in parts of Bihar and Orissa for over a century and it is more than likely that it existed in this country from the remote past.

The morphology and the intermediary hosts of the parasite, the histopathology and the symptoms of this peculiar schistosomiasis of cattle and buffaloes are now known. Still there are gaps in our knowledge regarding such points as other definitive hosts, if any, the regional distribution of the disease, the conditions governing such distribution, its therapy and control. In this paper the present authors record their observations regarding its therapy.

The earliest recorded account of the treatment of this disease is by Jeysing Raj [1908] who adopted surgical methods, for which purpose he invented two scoops. Theagaraj [1919] and Subramaniam [1919] attempted to treat such animals by rubbing a mixture of powdered copper sulphate and alum to the lesions in the nose without success.

Parthasarathi [1921] was the first in the field to adopt a successful method of treatment by injecting intravenously solutions of tartar emetic and this he did at a time when nothing was known about the etiology of the disease. This line of treatment was followed by Bhuvarahachari [1922] and others.

In the annual reports of the Madras Civil Veterinary Department, mention has been made from 1925-26 onwards of the number of animals successfully treated with tartar emetic. It would appear from records available on the subject that most of the work on the treatment of this disease with tartar emetic was done in the Madras Presidency, but the absence of information regarding deaths during the course of treatment is conspicuous. In the annual report of the Civil

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Veterinary Department, Madras, 1931-32, mention has been made of one case successfully treated with Antimosan. Roy Chowdhuri [1932] has also recorded his experience in the treatment of nasal schistosomiasis with this drug.

Perusal of the records on the treatment of this disease reveals several gaps, notably with respect to : (1) the minimum dose of tartar emetic to effect a cure, (2) the percentage strength of the solution of the drug for intravenous injections, (3) the length of the course of treatment, (4) relapses, if any, after treatment and (5) the mortality during treatment and its causes.

Experimental work on the treatment of Nasal Schistosomiasis

Some experiments were conducted in the Madras Veterinary College Laboratory during the year 1935-36 commencing in March 1935, and the scheme of work was so arranged as to ascertain :—(1) the efficacy of antimonium tartaratum, Antimosan and Trypaflavin in the treatment of bovine nasal schistosomiasis, (2) the minimum effective dose of each drug and the necessity for repetition to cure an animal, (3) the progress of recovery in the animals under treatment as ascertained by enumeration of ova in a measured quantity of nasal discharge during a reasonable period of time after the course of treatment, (4) whether any relapse occurs after a clinical or complete cure, in the absence of fresh infection.

Eleven bovines suffering from the disease, in varying stages of intensity, were obtained from Chittoor district on the 8th March 1935. The history of these cases showed that each of them had had the disease for a year or over. Their symptoms and the average number of ova in 0·1 c.c. of the nasal discharge were recorded for over a week before the commencement of treatment. It is interesting to note that those animals which had gross lesions showed smaller number of ova in the nasal discharge than those which had only ulcerations on a thickened mucous membrane. The animals were divided into three groups and each was treated with varying doses of the selected drugs at different intervals. This may not help an analysis from a statistical point of view, yet it gives definite information in regard to the most effective and the cheapest drug for the treatment in this condition. The treatment was commenced in all the three groups on the 18th March 1935 and the animals excepting those that died were under observation till the 18th August 1935.

Details of experiment

Group A.—This consisted of four animals and these were treated with tartar emetic in the form of a 6 per cent solution given intravenously.

> *Bullock No. 1.*—Weight 252 lbs. Age 6 years. Duration of disease one year. It had large lesions with intense snoring. The average number of ova in 0·1 c.c. of the nasal discharge was 17. It received a dose

of 3·75 grains of the drug (or 1·5 grains per 100 lbs. body-weight) on six consecutive days. The total quantity of the drug for the whole course of treatment was 22·5 grains. Daily examination of the nasal discharge showed that dark and dead ova began to appear from the fifth day after the commencement of the treatment, and the ova disappeared from the 12th day. In six weeks the snoring and the lesions disappeared. The animal improved in condition progressively and weighed 365 lbs. before it was destroyed on 13th August 1935. A careful post-mortem examination was held and no schistosomes could be detected in its nasal veins and the histological examination of its nasal mucous membrane showed that the lesions had healed.

Bullock No. 2.—Weight 308 lbs. Age 6 years. Duration of disease one year. It had small lesions and little snoring. The average number of ova in 0·1 c.c. of the nasal discharge was 5. It was given 9 grains of the drug (or 3 grains per 100 lbs. body-weight) repeated every fourth day and received in all 36 grains in two weeks. In the nasal discharge of this animal, dark and dead ova began to appear from the third day after the primary injection and no ova could be seen from the tenth day onwards. The other symptoms abated in 3 weeks. This case was cured and weighed 392 lbs. at the end of 5 months.

Bullock No. 3.—Weight 262 lbs. Age 4 years. Duration of disease one year. It showed ulceration on the tumified nasal mucous membrane with very little snoring. In the nasal discharge, 38 ova on an average, were found in 0·1 c.c. It was injected with 12 grains of tartar emetic per dose (or 4 grains per 100 lbs. body-weight). This animal had a rise in temperature within 48 hours after the injection (103·8° F.) and it returned to normal on the sixth day. This dose of the drug was repeated on the seventh day. Some hours later its temperature rose up to 105°F. and the animal died 12 hours after the injection. The cause of death was perhaps due to antimony poisoning. Post-mortem examination revealed dead schistosomes in the veins of the nasal mucous membrane and also in the clot of blood found in the heart. In this animal, from the fourth day after the primary injection, dark and dead ova began to appear in the nasal discharge and the total number of ova lessened by the sixth day.

Bullock No. 4.—Weight 308 lbs. Age 7 years. Duration of disease 2 years. It had gross lesions in the nose and snored intensely. About 28 ova were present in 0·1 c.c. of nasal discharge. This animal was injected with 15 grains of the drug (or 5 grains per 100 lbs. body-weight). Within 18 hours after the injection, its temperature rose up to

105·8° F. and it was dull and unable to feed or move. It died nearly 36 hours later. Post-mortem examination revealed a fairly large number of dead schistosomes in the veins of the nasal mucous membrane, in the clot of blood in the heart cavities and in the pulmonary arteries. The endocardium was inflamed in places and there was effusion in the pericardial sac. The mucous membrane of the abomasm and the small intestines was slightly congested in small patches in about 3 or 4 places.

Group B.—This group contained four animals which were treated with Antimosan, kindly supplied in the form of a 6·3 per cent solution by Messrs. Havero Trading Co., Ltd.

Bullock No. 5.—Weight 260 lbs. Age 12 years. Duration of disease 2 years. It had mild lesions in the nose, with intense snoring. There were, on an average, 42 ova in 0·1 c.c. of the nasal discharge. It was injected with 5 c.c. of the solution (or 2 c.c. per 100 lbs. body-weight) on alternate days till four injections were given. In all 20 c.c. (or roughly 18 grains of antimosan) were given. Dark and dead ova began to appear in the nasal discharge from the 4th day onwards. In the course of a fortnight, the symptoms abated and at the end of a month, ova disappeared. On the fifth week, ova reappeared though the animal looked apparently cured. It was then injected with antimony tartarate at 2 grains per 100 lbs. body-weight on alternate days until three injections were given. The ova disappeared and lesions healed completely in the course of three weeks. At the end of the period of observation, the animal was perfectly healthy and weighed 398 lbs.

Bullock No. 6.—Weight 280 lbs. Age 6 years. Duration of disease 2 years. This animal had large lesions and intense snoring. The average number of ova was 7 in 0·1 c.c. of the nasal discharge. It was injected with 12·5 c.c. (or 5 c.c. per 100 lbs. body-weight) twice a week, till four injections were given. In all, it received 50 c.c. (or roughly 48 grains) of the drug in two weeks. From the fifth day, dark and dead ova began to appear and all the symptoms disappeared at the end of the period of observation. The animal weighed 397 lbs.

Bull No. 7.—Weight 252 lbs. Age 4 years. Duration of disease one year. This animal showed small lesions with very little snoring. There were, on an average, 16 ova in 0·1 c.c. of the nasal discharge. It was injected with 18·75 c.c. (or 7 c.c. per 100 lbs. body-weight) weekly till two doses were given. In all it received 37·5 c.c. (or 36 grains of antimosan in two weeks). Four days after the first injection, dark and dead ova commenced to appear and all ova disappeared,

along with other symptoms, by the end of four weeks. Ova began to re-appear 18 weeks after the first injection. The animal was again treated with the same dose of antimosan which caused them to disappear in about ten days. The animal appeared clinically cured.

On 12th August 1935, this animal was selected to try the effect of progressively larger doses of tartar emetic. It was first given 3·75 grains of that drug per 100 lbs. body-weight in one dose. Three days later, it was given 5·6 gains per 100 lbs. body-weight and within 12 hours the animal died. This animal showed a rise in temperature to 103°F. within 24 hours after the first injection, was dull and off-feed during that period. On post-mortem examination, no schistosomes were found, but endo-carditis and congestion of the intestinal mucous membrane were noticed. It would appear, therefore, that a dose of 3·75 grains per 100 lbs. body-weight of the drug may produce a reaction in cattle and is unsafe to repeat it during the period of reaction.

Heifer No. 11.—Weight 196 lbs. Age 3 years. Duration of disease 1½ years. This animal was first treated with Trypaflavin and since no improvement was noticed, it was given Antimosan from the 25th April 1935. It showed large lesions and snoring was pronounced. Ten ova on an average were seen in 0·1 c.c. of the discharge. It was injected with 5 c.c. of the drug (or 2½ c.c. per 100 lbs. body-weight) every alternate day till three doses were given. No improvement was noticed during the four weeks after treatment. It was again injected in the first week of June, with 15 c.c. (or 7½ c.c. per 100 lbs. body-weight) every alternate day till three doses were given. At the end of four weeks the symptoms abated, and ova disappeared. There was no relapse till the 17th August 1935 when the period of observation terminated. The animal weighed 253 lbs. at the end of the experiments. It received in all, 15 c.c. or nearly 14 grains of Antimosan in the first instance and 45 c.c. or about 42 grains in the second course.

Group C.—This group consisted of three animals which were treated with Trypaflavin.

Bullock No. 8.—Weight 252 lbs. Age 7 years. Duration of disease 3 years. It showed about 10 ova on an average in 0·1 c.c. of nasal discharge. It was injected intravenously with 3 grains of Trypaflavin in 10 c.c. of water daily for 9 days. No improvement was noticeable during a period of 5 weeks and ova continued to appear. It is interesting to note that this animal developed acute hepatitis during

the course of treatment and became weak and emaciated. On the 26th April it was injected with 5 grains of tartar emetic. It reacted severely after this injection. The animal was dull, off-feed, and unsteady in gait though the temperature was normal. This condition lasted for five days. A week later, it was again injected with the same dose of tartar emetic.

Even this dose caused a reaction lasting for four days. Within three weeks after the last injection the ulcers in the nose and ova in the nasal discharge disappeared and the animal was found cured at the close of the experiments. Its weight was then found to be 325 lbs.

Bullock No. 9.—Weight 210 lbs. Age 4 years. Duration of disease 2 years. It showed small but numerous lesions on the nasal mucous membrane and snoring was not pronounced. The average number of ova in 0·1 c.c. of nasal discharge was 84. This animal was injected intravenously with 10 grains of Trypaflavin in 20 c.c. of water on the 18th of March, followed by one gram of the drug in a pint of water administered orally on the 20th, 22nd and 27th March. There was no improvement nor disappearance of ova and it suffered from hepatitis as in the previous instance. After observation for a period of seven weeks it was injected with antimony tartarate at 2½ grains per 100 lbs. body-weight on the 9th, 14th and 16th May. On the 13th May, dead and disintegrated ova began to appear and ova disappeared completely from the 17th May onwards. No lesions were to be seen in the nose from the 30th May. The animal was found to be cured at the end of the period of observation and weighed 338 lbs.

Heifer No. 10.—Weight 168 lbs. Age 2 years. Duration of disease 9 months. This animal had large lesions and intense snoring. There were on an average 9 ova in 0·1 c.c. of the nasal discharge. It was treated with one gram of Trypaflavin in a pint of water given as a drench daily till 5 doses were given. No improvement was noticeable during a period of 4 weeks. It did not develop hepatitis like the previous two, which received the drug intravenously. On the 26th April it was given an intravenous injection of antimony tartarate at 3 grains per 100 lbs. body-weight and this dose was repeated on the 29th April and again on the 1st May. The animal did not react to this dose and all the symptoms abated in about a fortnight later. This animal was found free from the disease and weighed 237 lbs. at the close of the experiments.

F

Discussion on the dosage of antimony tartarate and its dilution

Only very meagre information is available in text-books on Veterinary Pharmacology and Toxicology regarding the therapeutic dose of this drug and its dilution for intravenous use in cattle. Some amount of information is available, however, in papers on the treatment of flystruck cattle in Africa contributed by Hornby [1919, 1921]. He seems to have used 1·6 to 2 grains per 100 lbs. body-weight. Edwards [1929] commenting on the work of Das, says that at Muktesar, cattle tolerate doses of 5 c.c. of a 3 per cent solution (or 2·25 grains per 100 lbs. body-weight), that this dose can be repeated daily for about 10 days and that it is not safe to increase this dosage, though cattle may tolerate double that dose for three days. Even with 2·25 grains per 100 lbs. body-weight, Edwards says that accidents may follow. From our experiments, it is gathered that a dose which causes a reaction is a sub-lethal dose. Hence, the therapeutic dose should be much less than the sub-lethal dose and considerably so, if it is to be repeated at short intervals for the reason that the drug is more or less cumulative. Our experiments show that a dose of 1·5 grains per 100 lbs. body-weight, given daily for six days, is not harmful in any way and at the same time, the desired results are obtained.

From the published records of previous workers, it is not possible to calculate the doses they gave per unit of body-weight since none of them excepting Das, seems to have noted the weights of the animals treated. Doraiswami [1934] and Iyengar [1934] appear to be the only two, who computed the dose of the drug according to the body-weight of the animals they treated. The former gave 3 or 4 grains while the latter only 1·25 grains per 100 lbs. body-weight. Iyengar goes further in saying that the dose can be reduced to one grain per unit of body-weight without losing the efficacy of the treatment. Edwards [1928] found that the minimum lethal dose of tartar emetic to hill cattle is 14 c.c. of M-10 solution or just over 5·5 grains per 100 lbs. body-weight. Our experiments confirm this.

As regards the dilution of the drug for intravenous use in cattle, there have been differences of opinion among the previous workers. It would appear, that they used solutions ranging from 2 to 15 per cent in strength, presumably without any ill-effects. Krishnamurthi [1922] said that a powerful drug like antimony tartarate should be given in the form of a 2 per cent solution and administered 20 grains of the drug in that dilution, but the animal collapsed and died 12 hours later. Das [1929] injected 15·5 grains in 50 c.c. of water (nearly 2 per cent solution) in an animal weighing 440 lbs. (or 3·5 grains per 100 lbs. body-weight) and it died soon after. Pillai [1934] recommended a different plan of action. He chose to give 10 to 20 grains of the drug, presumably in 20 c.c. of saline or water (3·3 to 6·6 per cent solution) and injected it into the jugular vein, very slowly, taking over five minutes to complete the operation, so as to allow further dilution of the solution in the blood before it reached the heart. With this procedure, he says, he met with no accidents of any sort. It is doubtful, if such a length of time for

the operation is at all necessary. Much care, no doubt, is necessary to prevent the solution escaping into the perivascular tissue, the chances of which are obviously greater if five minutes are taken, instead of as many seconds, to complete the operation. In actual practice, it is not possible for one to take such a long time for each injection when many animals have to be done in a day. Hornby [1921] used a 4 per cent solution for intravenous injections and appears to have finished the operation rapidly with no ill-effects. Other workers such as Richardson [1928] and Holmes [1921] have also used a 4 per cent solution or even a little over, without any bad results, but the dose they all gave appears to be not more than 2 5 grains per 100 lbs. body-weight. In the experiments done here, a 6 per cent solution was injected fairly rapidly into the vein in the animals treated and no ill-effects followed. It has already been pointed out, that even 15 per cent solutions have been injected by the previous workers without any ill-effects. It, therefore, seems clear that it is not the dilution of the drug that causes accidents so much, as the dose and its repetition. A dilute solution may perhaps cause a less intensive tissue reaction than a more concentrated one, if by chance, it escapes into the perivascular tissue.

Elimination of tartar emetic from the system

Therapeutists admit that tartar emetic is a cumulative drug, though less so than arsenic. Clark [1932] says that antimony unlike arsenic is rapidly excreted and there is less tendency for cumulative poisoning to occur, when the drug is given intravenously. He further says that 30 per cent of the drug is excreted through the urine and it is not known how much is excreted in faeces when it is given intravenously. Faust and Maleney [1924] say that a large proportion of the drug is excreted by way of the intestines. Edwards [1928] says that the drug is not discernible in the blood stream three days after intravenous administration. Krishna Iyer and Sarwar [1935] say that in two days time, it is excreted completely. Hence, it is clear that a therapeutic dose given intravenously is excreted rapidly and theoretically speaking, it should be possible to repeat this dose in three days. But practical experience has shown that death may be caused, if the same dose is repeated after a reaction has been obtained to the first dose. Such a thing did happen in Bullock No. 3, even when the interval between the two doses was a week and also in Bull No. 7. The symptoms of reaction observed in the experimental animals were (1) a rise in temperature, (2) disinclination to move, (3) weakness of pulse, (4) loss of appetite, (5) dryness of the mucous membrane of the mouth and nose, and (6) unsteadiness of gait. These symptoms persisted for about four days. If these symptoms are encountered in an animal after injection of the drug, it indicates that the limits of a therapeutic dose have been transgressed and those of a sub-lethal dose have been reached. In such cases, if the dose is repeated before or soon after the signs of reaction have subsided, accidents are likely to occur : at any rate, it is an indication, that subsequent doses should be smaller in order to avoid untoward results.

It is interesting to note that Parthasarathi [1921] considered that the proper dose of the drug should be such as to cause a reaction in the animal and the symptoms he describes are almost the same as those met with here. In the absence of reaction to a certain dose, he advocated the repetition of an increased dose, the very next day until a reaction was set up. He said that this reacting dose, which in his opinion was the dose of maximum therapeutic efficiency could be repeated at intervals of two to seven days according to the degree of severity of the disease. It is evident from this that Parthasarathi treated his cases with sub-lethal doses of tartar emetic. It is unfortunate that neither he, nor other workers who adopted his method of treatment reported cases of mortality, if any, during treatment, perhaps the deaths that occurred were not brought to their notice or ascribed to some other cause. It seems likely that some deaths would have occurred as a result of pushing sub-lethal doses without allowing sufficient time for excretion of the drug from the system. Krishnamurthi [1922], Mahadevan [1923] and Das [1929] are the only workers who have recorded deaths in animals treated with tartar emetic. Obviously, therefore, reacting doses and their repetition at short intervals are not safe in the treatment of cattle suffering from this complaint.

The action of the drug on schistosomes and their ova

Faust and Meleney [1924] ascertained how quickly schistosomes are killed *in vitro* in various concentrations of sodium-antimony-tartarate. They found, that in a dilution of 1 to 42,000 the worms died in one hour. They, therefore, opined that if this concentration is exceeded in the body, for even a short space of time after the administration of the drug, and if such a dose is repeated daily or on alternate days, the worms would soon be killed. Their work gives us a clue on which to base the lowest daily dose of tartar emetic sufficient to kill the worms without causing harm to the animal treated.

In our experiments, the lowest dose administered was 1·5 grains per 100 lbs. body-weight, which represents a dilution of 1 to 32,000 in the blood of an animal at the time of injection. This concentration is more than the limit laid down by Faust and Meleney. The repetition of this dose daily, for a reasonable length of time, should, therefore, kill the worms. It was found in experimental animal No. 1 that six such daily injections cured the animal and this was verified at autopsy nearly four and a half months after treatment. The drug has action on the ova of schistosomes in the tissue, which is borne out by the fact, that in about four or five days after the commencement of treatment, dark and dead ova appeared. Hence it would seem that successful treatment of a case of schistosomiasis does not so much depend on the total amount of the drug administered as on the maintenance of a certain amount of concentration of the drug in the blood at a constant level by repeated injections of suitable doses.

Obviously, therefore, it is of little use either to give a reacting dose or to increase the dose progressively at long intervals, as suggested by many of the previous workers in the treatment of this disease.

Length of the course of treatment.—The length of the course of treatment depends on the number of injections to be given and the interval between them. The records of previous workers show that the length of the course of treatment adopted by them varied from four to fourteen weeks. It is seen that they gave fairly large doses of tartar emetic at long intervals which naturally increased the length of the course of treatment. It has been pointed out above that the drug is fairly rapidly eliminated, when given intravenously; hence it would appear that when the interval between the injections is long, the level of the required concentration of the drug in the blood is not maintained for a sufficient length of time to kill the worms. Such animals may appear to have been cured, but relapses occur sooner or later. This may be one of the reasons why some of Oxpring's [1932] animals relapsed after an apparent cure and a second and even a third course of treatment was found necessary for them, thus extending the course of treatment over several months. He gave 10 grains of tartar emetic per dose, irrespective of the body-weight of the animal treated, once in four days, till ten doses were given to each animal.

Das [1929] treated some cattle in six villages with intravenous injections of 10 to 20 grains of tartar emetic. He found that the cases that had five injections were either cured or improved but those treated with fewer doses did not appear to have derived any lasting benefit. With the experience gained, he opined that massive doses may not have curative effect, and said that a sound plan would be to inject 10 to 12 grains of the drug at less than four days' interval and when smaller doses are employed, the interval may be reduced to one day. To reduce the length of the course of treatment or the number of injections, some workers suggested increased dosage, with a margin of safety, to be given at short intervals either because the owners fail to present their animals regularly to complete the full course of treatment or because it is a long and laborious task to do a large number of injections in one day, if many animals have to be done. Theoretically speaking, this method may seem to be sound because such doses should kill or disable the schistosomes shortly after the treatment is begun, thus giving a greater chance of a cure even if the animal is not subjected to the regular course. But in practice, the large dose, either progressively increased or otherwise if given at shorter intervals, may kill the animal itself while under treatment for reasons already discussed above. Most of the previous workers employed the so-called reacting or sub-lethal doses and advised the ryots to rest their animals at least for some time before they worked them. Some employed a routine method of administering 10 grains of tartar emetic irrespective of the body-weight of the animal treated, and even among these, reactions have been observed. Ramaswami [1933] in a communication to one of us, says that he gave 10 grains of tartar emetic

to each animal while conducting mass treatment against bovine nasal schistosomiasis in Chittoor district, and that he found many of the animals reacting to this dose possibly because a large majority of cattle are small in size in that district. Some of the ryots worked their animals in the plough soon after injections of tartar emetic, and to their dismay some of them dropped down dead while working. In such animals evidently there was a reaction set up and they died of its effects aggravated by work. The ryots, in most villages, being poor, do not possess more than one pair of bullocks; necessarily they cannot afford to rest their animals since they have none others to do the work. If, therefore, such disasters were to be anticipated, the ryots even with persuasion, for obvious reasons, will not subject their animals to treatment. Therefore, one has to employ such doses as would not cause reaction; in other words, the doses employed must be small so as to be given repeatedly with impunity, even if the animals are worked immediately during treatment.

It is seen from the experiments done here that a daily dose of 1·5 grains per 100 lbs. body-weight for six days is not only effective but also reduces the length of the course of treatment when compared with any other method. Further, there is no possibility of producing a reaction with such a small dose, thus avoiding the danger of the animal collapsing and dying.

Discussion on the treatment with Antimosan

Antimosan is a synthetic antimony preparation recommended by the makers for use in the treatment of schistosomiasis in cattle. The drug is available in the form of 6·3 per cent solution and the dose recommended is 40 c.c. for an animal weighing 450 lbs. or 8·8 c.c. per 100 lbs. body-weight. This dose, they say, should be repeated every second or third day. 100 c.c. of this drug contains only 12·5 grains of antimony: hence in the dose recommended there are only 5 grains of it or roughly 1·2 grains per 100 lbs. body-weight. It is interesting, therefore, to note that this dose of antimony is nearly equal to that of antimony tartarate which the present writers found to be safe and efficacious, for daily injections, for six days.

The makers of antimosan quote Roy Chowdhury [1932] regarding the usefulness of the drug in the treatment of bovine nasal schistosomiasis. That worker was supplied with the powder form of the drug. It would appear that he did not compute the dose of the drug per unit of body-weight, but gave varying doses, which, in some cases were increased progressively with an interval of a week between two doses. Hence, it is not possible to compare his results with those obtained by the present writers. It will be seen that the doses he gave were smaller and spaced at longer intervals than those recommended by the makers of the drug. The present writers found that in an animal treated with small doses of Antimosan, the symptoms abated in about 4 weeks. The animal looked clinically cured, but this was not the case, as ova continued to appear. Hence, the

results of Roy Chowdhury [1932] cannot be said to be conclusive in as much as the animals he treated with Antimosan were not under his observation for a sufficiently long time, nor was it possible for him then, to appreciate the results of treatment by laboratory methods as can now be done, since the etiology of the disease is known. Roy Chowdhury opines that recently infected animals require only one dose for a cure. It is doubtful, if, in the treatment of this disease, the administration of a single large dose of any drug will effect a cure and the reasons are obvious. Another interesting observation is that recovery was definite in those animals which received, at short intervals, doses almost equal to those recommended by the makers of the drug. It would appear, therefore, that the proper dose of the drug is the one that has been recommended by the makers repeated at intervals of 2 or 3 days until about 4 injections are given.

Discussion on the Treatment of Trypaflavin

Trypaflavin is the same as Acriflavin. This drug was tried, because Fisher [1934] reported, that he obtained with Acriflavin, some amount of success in the treatment of intestinal schistosomiasis in the human being. He gave the drug orally and no toxic manifestations seem to have been developed.

Trypaflavin was found to be useless in the treatment of bovine nasal schistosomiasis and on the other hand, it was noticed that the drug when given intravenously for some time, has harmful effects on the liver.

Cost of treatment with tartar emetic and Antimosan

The cost of a pound of tartar emetic is about Rs. 1-8-0 and the cost of the full course of 6 injections for an animal weighing 450 lbs. at 1·5 grains per 100 lbs. body-weight is 1·5 pies. This drug, therefore, is so cheap that nearly 200 animals of average weight may be treated at a cost of Rs. 1-8-0, when compared with that of Antimosan which is about Rs. 2-8-0 for one animal for a course of treatment recommended by the makers.

SUMMARY

1. Antimonium tartaratum, Antimosan and Trypaflavin were given a trial in treating cases of bovine nasal schistosomiasis.

2. Trypaflavin was found to be useless. Antimosan appears to be safe and effective if given according to the instructions of the makers, but expensive.

3. Antimonium tartaratum is effective and cheap. The dose recommended is 1·5 grains for every 100 lbs. body-weight of the animal repeated daily for six days, or 2·5 grains every alternate day.

4. Any dose exceeding 3·5 grains per 100 lbs. body-weight seems to be dangerous particularly, if repeated.

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G

ON THE IDENTITY OF THE NEMATODE WORM RECOVERED FROM HUMP SORE OF CATTLE IN INDIA

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INTRODUCTION

In a preliminary report [1935] by the writer on the etiology of this disease it was stated that the adult nematodes recovered from the lesions bore certain resemblance to the members of the sub-family SETARINAE of the family FILARIDAE. The exact identity of the worm at that time was attended with some difficulty, since by the appearance of the worm and the nature of the lesion it sets up, it appeared to be a new parasite for which no reference could be found in older text-books on helminthology. During the earlier investigation of this disease when its etiology was known in 1933, the writer was informed by the Director, Imperial Institute of Veterinary Research, Muktesar, that the nematode larvae had also been seen in an identical affection of cattle called " Cascado " in the Dutch East Indies. No fuller reference on " Cascado " could be available until Datta in January, 1935, at the Indian Science Congress at Calcutta stated that his studies on the histopathology of hump sore had led him to conclude that this disease of Indian cattle was similar to " Cascado " of cattle in the Dutch East Indies, caused by STEPHANOFILARIA DEDOESI (Bubberman, C. and Kraneveld, F. C.). Few specimens were therefore received through the courtesy of Dr. F. L. Huber, Director, State Veterinary Institute, Buitenzorg, Java. On the result of a comparative study of the parasites of hump sore and " Cascado " the writer was able to ascertain that the parasite of hump sore belonged to the genus STEPHANOFILARIA, but that in certain morphological details it differed from STEPHANOFILARIA DEDOESI. In June, 1934, Chitwood described STEPHANOFILARIA STILESI, a new species from the skin of cattle in the United States. On the basis of Chitwood's description of STEPHANOFILARIA STILESI, many points of difference have been noticed between this worm and that recovered from hump sore.

In view of these observations it was decided to create a new species STEPHANOFILARIA ASSAMENSIS for the parasite of the Indian disease, by virtue of its being discovered for the first time in Assam. In the following pages it is proposed to give an account of the new species, and then comparing it with two other known species, to indicate certain anatomical differences which led to its specific diagnosis.
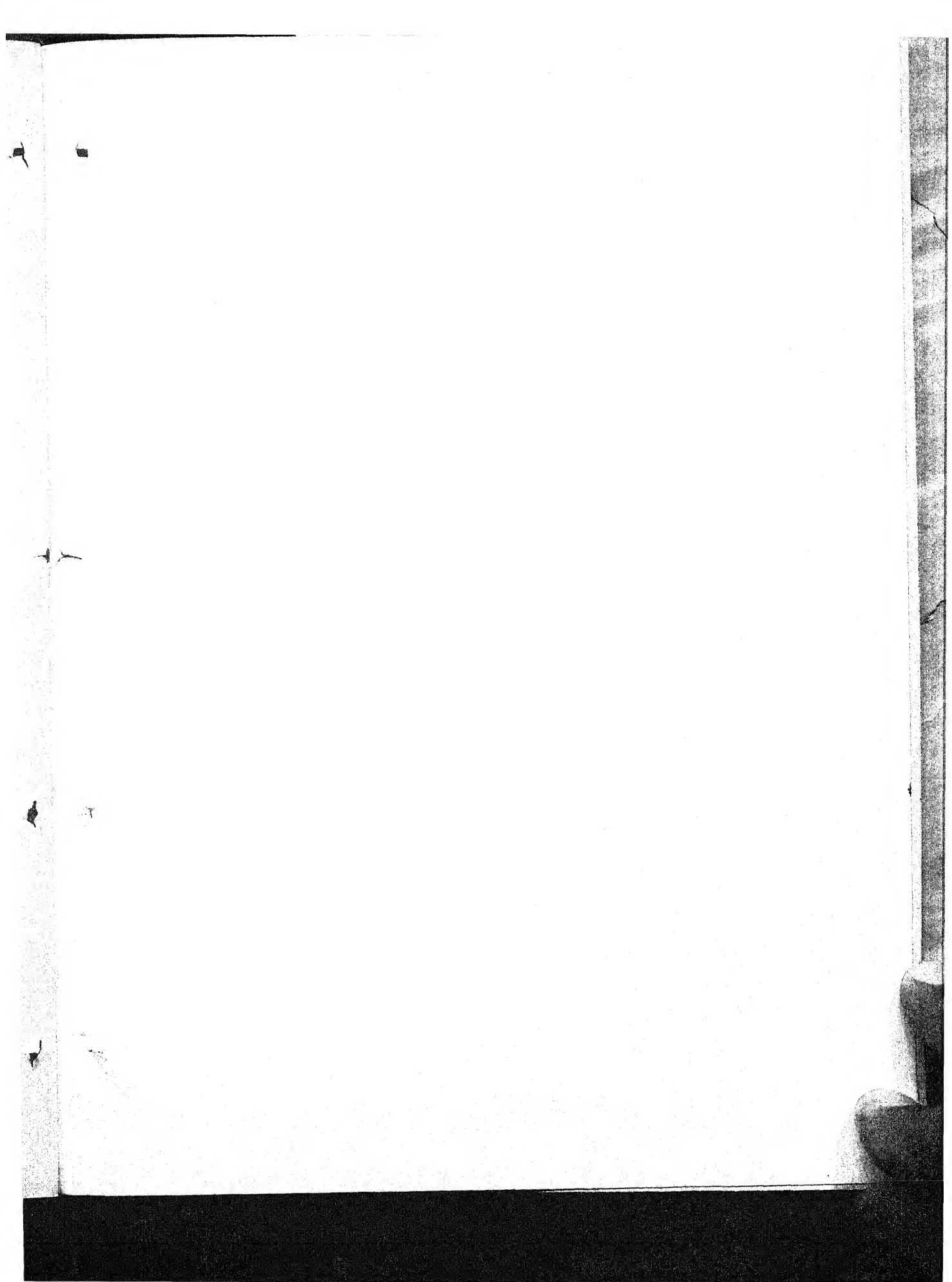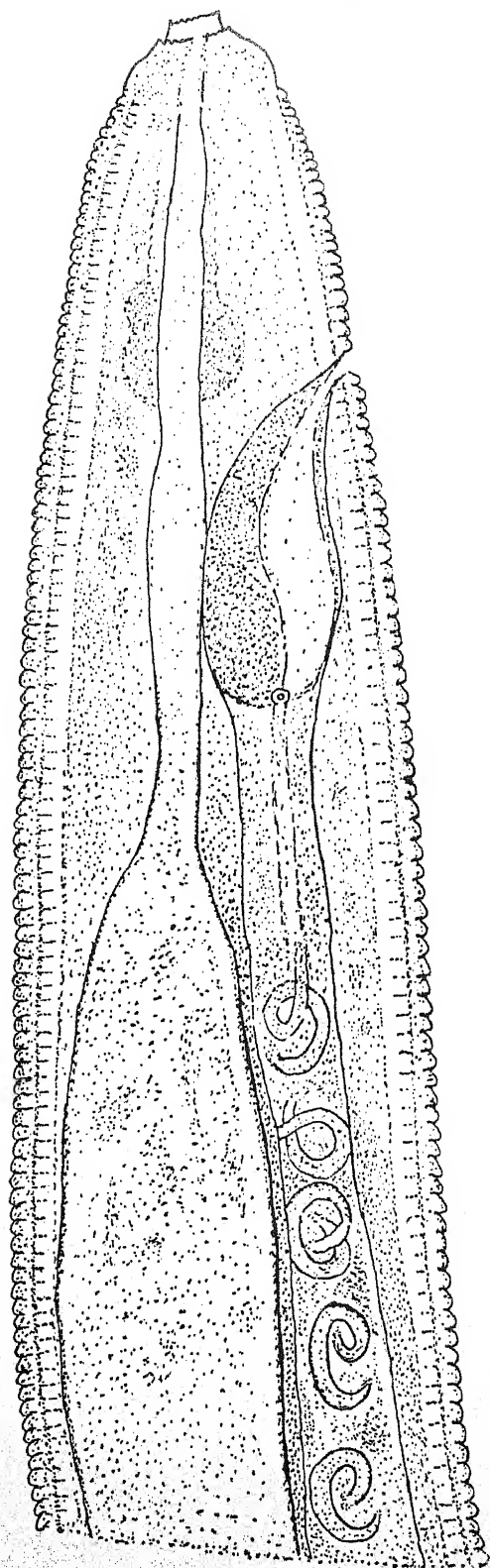
Fig. 2
400×
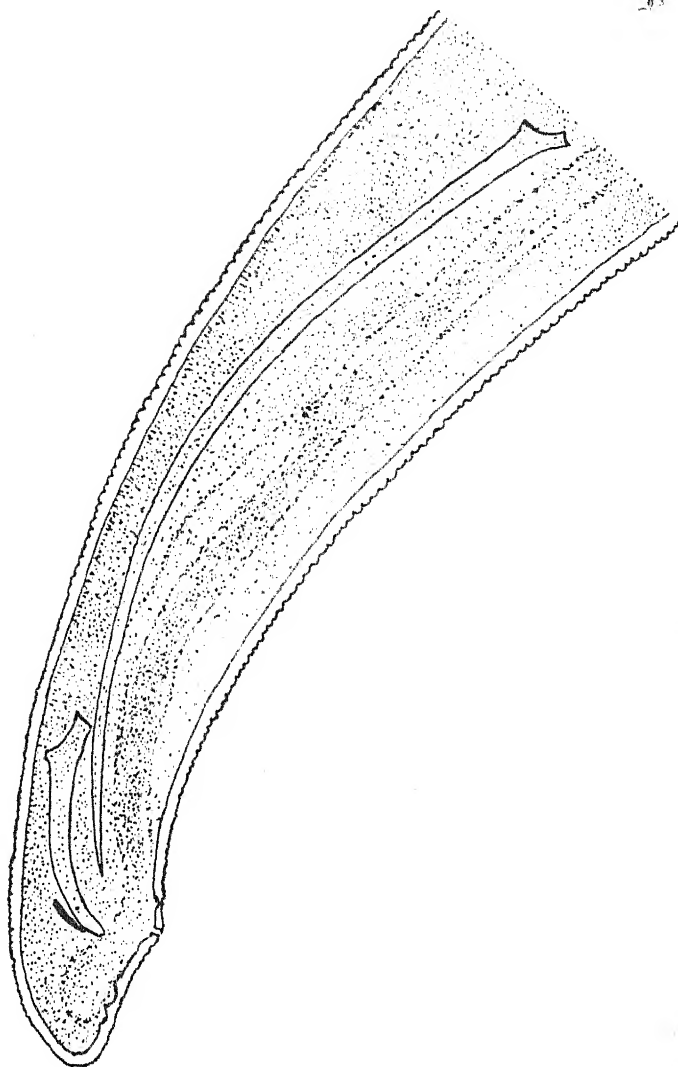
FIG. 1.   The anterior portion of the ♀ worm showing mouth
opening, vulvar opening, vagina, tubular oesophagus
and the nerve ganglia.

FIG. 2.   The posterior portion of the ♂ worm showing the
slightly curved extremity, anal opening, spicules and
the gubernaculum.

FIG. 1, 400×

TECHNIQUE

The collection of worms from the lesion of hump sore presents certain difficulties. The most convenient site for their recovery is the demarcation line between the central core and the periphery of a quiescent lesion. A place over this line is selected and the sore tissue scraped off with a slightly blunt scalpel. On thus scraping away the whole cornified layer of the superficial epithelium and a part of the rete malpighi, a thread like projection of what appears to be one of the ends of the female worm is seen on the surface. This is then picked up with a pair of finely pointed forceps and placed in a petri dish containing normal saline. The scraped up material should be placed in another petri dish containing normal saline, because not infrequently an entire female worm could be found embedded in a lump of scraped up tissue. The male worms are very minute, and for the purpose of their collection the scraped up tissue is thoroughly teased with a fine pair of needles, and a search is made for the parasite under one-third objective of the microscope. For the purpose of an immediate examination the embryonated ova could be collected by teasing a gravid female on a slide in a drop of normal saline. Plenty of ova are thus liberated either individually or in lumps which represent parts of the uteri. The saline film on the slide is then allowed to dry up so as to fix up the ova. The preparation is then stained with Leishman or Giemsa stain.

Certain anatomical features such as the cuticular spines, the vulva and the vagina could be seen best in a living specimen. Beechwood creosote has been used as a clearing agent to see various other details, such as the nerve ganglia, tubular oesophagus, and the spicules in the male.

A GENERAL DESCRIPTION OF THE PARASITE

Freshly recovered parasites from the sore have frequently been found covered with fragments of sore tissue towards their anterior extremity. The female parasites have always been found predominating over the male ones in number, and in collections made from several cases the numerical ratio between the male and the female has been 1 : 3. When placed in normal saline, the worms exhibit sluggish movements. They are small, whitish and thread like worms, the females being two to three times larger than the males. The anterior extremity of the male and the female which carries the mouth is slightly attenuated. The mouth opening (Plate XXIII, Fig. 4) is minute, circular and bordered with a notched chitenous ring. Immediately behind the mouth is a prominent conical structure (Plate XXII, Fig. 1, and Plate XXIII, Fig. 4) from which the mouth appears to be protruding anteriorly. At the base of the oral protrusion the thick conical structure is encircled with a single complete ring of cuticular spines (Plate XXIII, Fig. 4) which in some specimens also shows group arrangement at places. The cuticle is smooth over the conical structure, and commencing with a deep notch

behind the latter, it is transversely striated (Plate XXII, Fig. 1) throughout the body. For a greater length of the anterior part of the body, the posterior edges of the striae are directed posteriorly in the form of spines, but towards the posterior extremity the striae become inconspicuous and their posterior edges smooth. Lateral alae are present.

The terminal mouth leads into a tubular oesophagus (Plate XXII, Fig. 1) which measures about from 124 to 180 microns from the mouth opening in both the sexes. The nerve ring (Plate XXII, Fig. 1) in both the sexes is situated at a distance of 72 microns from the mouth opening. Cloacal opening is distinct in the male, (Plate XXII, Fig. 2) but it appears to be absent in the female.

The male worm.—The male parasite is 3 to 4·5 millimeters* long and 108 to 126 microns wide. The caudal extremity (Plate XXII, Fig. 2) is attenuated and slightly bent towards its ventral aspect. The alimentary tract widens out towards the posterior limit of the oesophagus in the form of a straight tube which constitutes the intestines. The intestinal tube ends in a minute anus opening into a cloaca which also receives the structures of the male genitalia. The latter consists of a single tubular testis, and two unequal spicules. The testis begins about the anterior fifth of the body, and runs parallel to the intestine. It is a compact structure consisting of cells which give rise to spermatozoa. Of the two spicules (Plate XXII, Fig. 2) the left one is the larger and measures about 150 to 180 microns in length. The right spicule being the smaller of the two measures about 43 microns. Near the smaller spicule and dorsal to it lies the small gubernaculum. The cloacal opening is situated at about 25 to 30 microns from the posterior extremity. There are two pairs of post-anal papillae (Plate XXII, Fig. 2).

The female worm—The female parasite is stouter and longer than the male. It measures from 7 to 9·5 millimeters in length, and from 190 to 208 microns in width. The caudal extremity of the female is broader than the oral extremity, (Plate XXII, Fig. 1, and Plate XXIII, Fig. 3) and is straight and stumpy. As in the male, the intestine begins from the posterior limit of the oesophagus as a straight tube like structure. For a certain distance in the anterior half of the worm, the intestine runs parallel to the vagina and the uteri, (Plate XXII, Fig. 1) but posteriorly (Plate XXIII, Fig. 3) it becomes indistinct in the loops of the uteri and the ovaries. No distinct anus has been observed. The vulva (Plate XXII, Fig. 1) which is a sack like structure measures about 72 microns in length, and opens out in the form of a spout at a distance of 75 to 90 microns from the mouth opening. Behind the vulva is the vagina which is a straight tubular organ measuring from 252 to 288 microns in length. Behind the vagina begin the two

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* Except for the length of the worms which is given in millimeters, all other measurements are in microns.
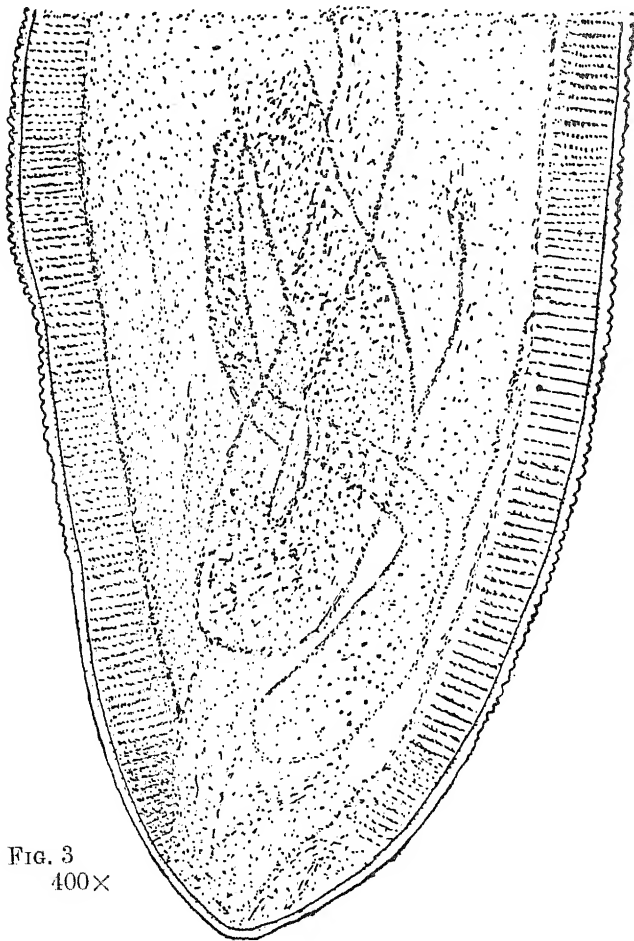
FIG. 3
400×

FIG. 3. The posterior extremity of the worm showing the coils of the uteri.
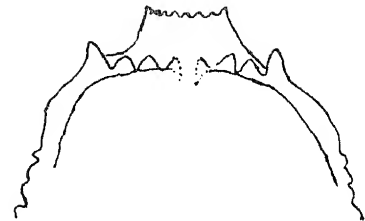
FIG. 4
900×

FIG. 4. Showing the notched rim of the mouth and the cuticular spines behind it.

FIG. 5
400×

6 (b)

6 (a)

FIG. 6
900×

6 (c)
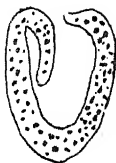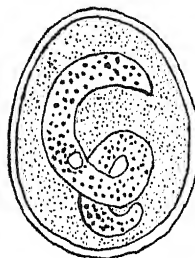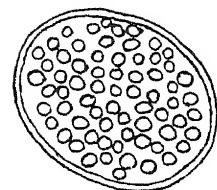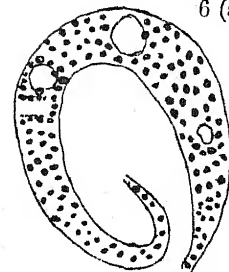
FIGS. 5 & 6. Showing microfilariae inside and outside the membrane.

uteri which run parallel for a certain distance, and then become indistinct in the ovarian coil situated posteriorly. In a gravid female worm the uteri and the vagina contain fully developed embryos (Plate XXII, Fig. 1) each enclosed in a flexible vitelline membrane. The embryonated eggs (Plate XXIII, Figs. 6 (a) and 6 (b)) measure from 39·6 × 28·8 to 43·2 × 36·0 with the membrane. The microfilariae as seen outside the membrane in a stained smear measure from 126 to 144 microns.

DISCUSSION

Ihle and Ihle-Landenberg [1933] were the first to describe the filarial worm recovered from "Cascado" of cattle in the Dutch East Indies, for which they created a new generic name STEPHANOFILARIA, and named the parasite as STEPHANOFILARIA DEDOESI. Wehr [1935] in his revised classification of the superfamily FILARIOIDEA has included a new family STEPHANOFILARIIDAE for the reception of the genus STEPHANOFILARIA, Ihle and Ihle-Landenberg [1933]. Another species of this genus has been described by Chitwood, [1934] as STEPHANOFILARIA STILESI. Chitwood distinguishes his species from the original species by the following two characters :—

1. The arrangements of the cephalic spines, and
2. The distance of the vulvar orifice of the female from the mouth opening.

The cephalic spines as observed by Chitwood [1934] in his species are incomplete and asymmetric, whereas in the original species they are apparently complete and symmetric. The writer while studying his new species has found the cephalic spines completely encircling the base of the oral elevation in some of the specimens, while in others, although the spines have been found in groups, their symmetric arrangement does not appear to be lost. Although Chitwood, for the description of his species, considers the arrangement of the spines as a distinguishing feature, he attaches no particular importance to it from the taxonomic standpoint. Certain important factors are likely to be involved in consideration of these spines as having any taxonomic value. It is to be remembered, however, that the species of STEPHANOFILARIA have so far been found to be distinctly pathogenic for the bovine skin where their presence induces a very extensive histological changes. The causation of the lesion by these worms depends to a certain degree on the irritation caused by the constant movements of these spines in the tissue of the skin. The arrangement of the spines, therefore, cannot remain a constant feature when they, in their chronic attempt at destruction of the skin tissue, are subjected to a severe strain of tissue reaction. In view of these observations, however, it is to be presumed that under a pressure of excessive tissue reaction the cephalic spines may either be pressed to lie in groups, or some of them may so completely be detached as to give the remaining ones an asymmetric

arrangement. In the opinion of the writer, therefore, the arrangement of the cephalic spines appears to have no value in the classification of the species of STEPHANOFILARIA. The other character, *i.e.*, the position of the vulvar orifice from the mouth opening by which Chitwood distinguishes his species from STEPHA-NOFILARIA DEDOESI appears to be an important feature in the taxonomy of the worm. Before any opinion is given as regards the identity of the worm at the disposal of the writer, it will be of interest to compare the salient features of the two known species with those of the species under consideration.

TABLE 1

Statement showing the points of difference amongst the three known species of Stephanofilaria

Morphological Characters	Stephanofilaria dedoesi, Ihle, and Ihle-Landenberg, 1933	Stephanofilaria stilesi, Chit-wood, 1934	Stephanofilaria assamensis, Pande, 1935
Male			
Length	2·3 to 3·2	3·0 to 3·5	3·0 to 4·5
Maximum thickness	70 to 90	40 to 50	108 to 126
Size of the left spicule	226 to 230	276	150 to 180
Caudal end	Straight	Slightly bent	Slightly bent
Position of the cloaca from the posterior end	22 to 32	..	25 to 30
Female			
Length	6·1 to 8·5	5·64 to 5·8	7·0 to 9·5
Maximum thickness	156 to 172	100 to 117	190 to 208
Position of the vulva from the mouth opening	49 to 57	78 to 90	75 to 90

It will be seen from the above table that both the male and the female of the Indian species differ from the other two known species in being a stouter and a comparatively bigger parasite. While there is not much difference in the length of the male of the three species, they show marked variation in the measurement of their thickness. The position of the cloaca in STEPHANOFILARIA DEDOESI and STEPHANOFILARIA ASSAMENSIS in which this feature has been observed shows no variation at all, but the left spicule of STEPHANOFILARIA ASSAMENSIS is much smaller than the same structure of the other two known species.

While the female of the Indian species is only slightly greater in length than the female of STEPHANOFILARIA DEDOESI, it is much greater in length when compared with the female of STEPHANOFILARIA STILESI. The position of the vulvar

orifice is almost the same in STEPHANOFILARIA STILESI and STEPHANOFILARIA ASSAMENSIS, and in this respect they both differ from STEPHANOFILARIA DEDOESI in which the opening of the vulva is at a much lesser distance from the anterior extremity. The Indian species can, therefore, be distinguished from the other two known species by the following features :—

1. The measurement of length and thickness.

2. The size of the left spicule, and

3. The position of the vulvar orifice from the mouth opening.

SPECIFIC DIAGNOSIS

Stephanofilaria :

Male.—Length 3·0 to 4·5, thickness 108 to 126, size of the left spicule 150 to 180, and the caudal extremity slightly bent.

Female.—Length 7·0 to 9·5, thickness 190 to 208, anus indistinct, position of the vulvar orifice at a distance of 75 to 90 microns from the anterior extremity.

Host.—Cattle.

Location.—Skin, beneath the rete malpighi.

Locality.—Assam (India).

The writer desires to express his thanks to Dr. F. L. Huber, Director, State Veterinary Institute, Buitenzorg, Java, for supplying to him few specimens of STEPHANOFILARIA DEDOESI, to the Director, Imperial Veterinary Research Institute, Muktesar, for lending him certain reference books on Stephanofilariasis, and to the Superintendent, Civil Veterinary Department, Assam, for his keen interest in the study of this parasite.

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DETOXICATED URINE TREATMENT FOR IMPOTENCY IN STUD BULLS AND STERILITY IN COWS

BY

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(With Plates XXIV & XXV)

When the senior author investigated in 1931 the causes of the gradually increasing number of cases of impotency in bulls and sterility in cows in the Pusa Sahiwal herd, he found that most of them were due to the faulty feeding policy pursued in the past. The rationing was, therefore, entirely changed [Sayer, 1934] and its excellent effect on both bulls and cows was soon apparent.

The few cases of impotency in bulls and sterility in cows that were not, however, amenable to the changed rationing were put under the hormone treatment [Phillip, 1929].

A number of cases of impotency and sterility were given the above treatment quite successfully, out of which two cases are quoted below for illustration. The method followed here is given below :

DETOXICATION OF URINE FOR INJECTION

Collect in a sterile enamel mug about 100 c.c. of urine from a cow over seven months in calf. Early morning urine is preferable.

Detoxicate at once.

To each 100 c.c. of urine add 5 c.c. of a 20 per cent solution of sulpho-salicylic acid.

Shake well for ten minutes to precipitate all proteins.

Allow to stand and pass through a coarse filter paper.

Neutralise or render faintly alkaline to litmus by the addition of bicarbonate of soda.

Dosage.—10 c.c. of urine per 100 lbs. body-weight injected subcutaneously behind shoulder daily on four consecutive days. Repeat in three weeks time, if necessary.

PLATE XXIV
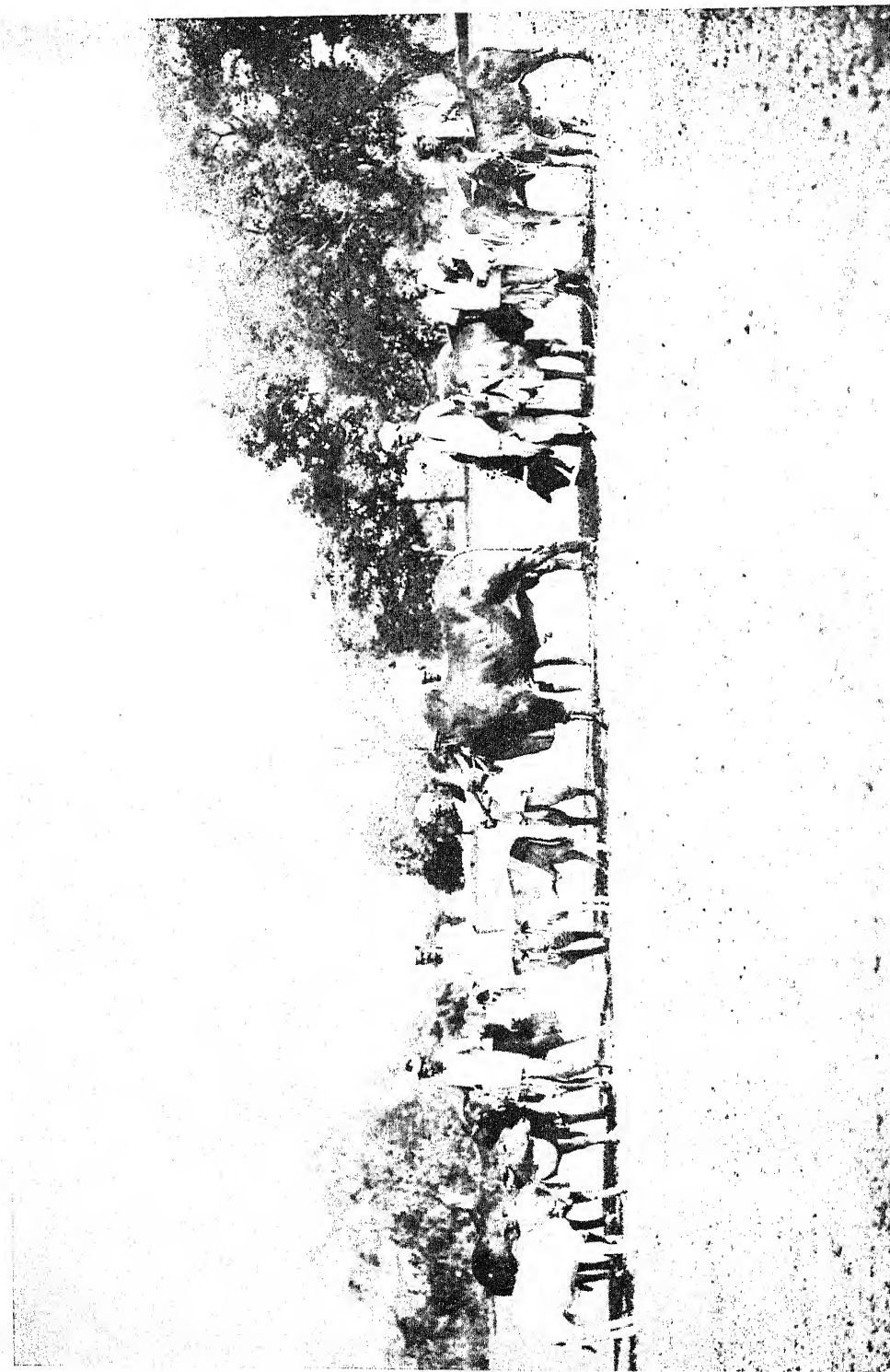
A rejuvenated Sahiwal bull of the Pusa pedigree herd with his female progeny.

Left to right :—

1. Choki (25-9-35)
2. 745 (Lachrama)
3. 735 (Chandrama)
4. 716 (Sampati)
5. Bripla (2-12-35)
6. Maharaj.
7. 706 (Lachrama)
8. 738 (Bripari)
9. 743 (Birbntia)
10. Noshni (16-8-35)

Ind. Jour. Vety. Sci. & Anim. Husb., Vol. VI, Pt. IV.
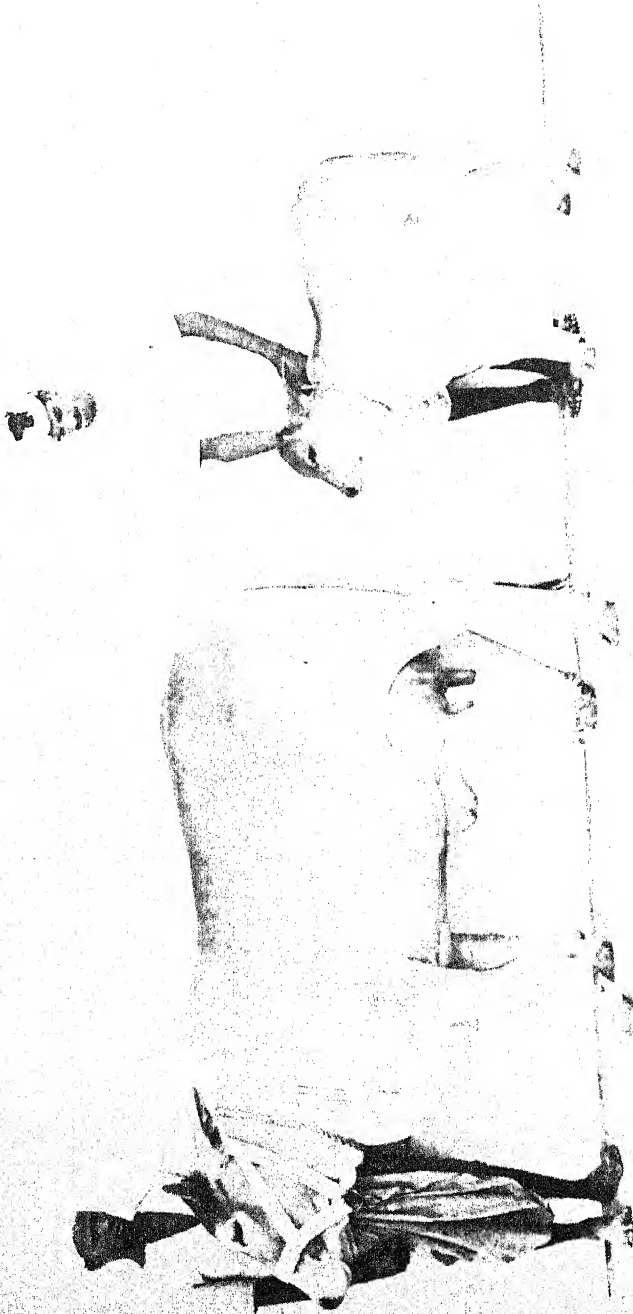
PLATE XXV

A case of temporary sterility treated with detoxicated urine from pregnant cows.

Her Cow Calf
(Born 15-2-36).

Lachmohni No. 644
(Born 10-9-31.)

SAHIWAL BULL 'MAHARAJ' No. 300 (PLATE XXIV)

This bull was born on the 27th January, 1920, of Raseeli No. 132, one of our foundation cows, by Sahiwal bull Mansoor. His dam was a typical Sahiwal cow with a natural disease-resistant constitution, who gave fourteen calves and 30,000 lb. of milk during her life time on the Farm. She lived twenty-two years without any serious ailment and was a regular calver. It was decided to keep this bull for stud purposes.

When Maharaj was three years old, he was tried with a number of cows for service and failed utterly ; consequently he was sent uncastrated to the Agricultural Section for draught purposes.

In 1932, he was brought back from the Farm and given the " detoxicated urine treatment " and since then he is serving satisfactorily and so far he has sired twenty-two calves, out of which eleven are heifer calves. His disease-resistant faculty was tested this year during the outbreak of Foot and Mouth disease. He was artificially infected with the infective saliva thrice, but he proved absolutely immune and similar tests with his progeny also showed them naturally resistant to the disease to a great extent.

His first heifer which was born on the 23rd December 1933, received bull when she was one year, six months and thirteen days old. She has since calved, developed a very good shape bag, and has given 2,122 lb. of milk in 107 days and is giving about 16 lb. daily.

The average birth weight of his calves is 43·3 lb. and this shows that the progeny he is producing is quite normal and there is not the least trace of lack of stamina or reduction in size and conformation.

SAHIWAL COW 'LACHMOHNI' No. 644 (PLATE XXV)

She calved for the first time on the 7th July, 1934, and did not come in oestrus (heat) after the usual period of 150 days—time allowed for heavy milkers on this farm for bulling. She was examined and finding the reproductive organs normal ; she was given the above treatment on the 2nd April 1935, and she came on heat and received bull on the 2nd May, 1935. She completed her first lactation on the 16th May 1935, with 5,191 lb. of milk in 306 days.

She calved again on the 15th February, 1936, has given 5,238 lb. of milk in 172 days and is giving about 24 lb. per day.

Several similar cases have been successfully treated on this farm with the detoxicated urine from pregnant cows.

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SELECTED ARTICLE

STUDIES ON BOVINE MASTITIS

XI.—FURTHER OBSERVATIONS ON THE CONTROL OF CHRONIC STREPTOCOCCUS MASTITIS

BY

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In 1933 we gave an account of a herd from which chronic streptococcus mastitis due to *Str. agalactiae* had been eradicated by the segregation and eventual sale of infected cows. This confirmed the generally accepted belief that this disease is strictly contagious and that, therefore, it should be possible to control it by the simple procedure of avoiding direct transmission during milking. At that time similar control measures were also being carried out in six other herds, *viz.*, Herds A, C, I, J, K and L and the purpose of this report is to detail the results of this work.

It is evident from the literature on the subject that laboratories concerned with mastitis are now giving more attention to control of the disease by segregation, with or without treatment of infected cows. Vaccines, autogenous or other, have gradually fallen into disrepute because, although some authors believe they reduce the more obvious manifestations of the disease, they do little or nothing to diminish the number of infected udders or to prevent the spread of the disease. In accordance with this view, many workers who have tried vaccination, *e.g.*, Seelemann, Plastridge, Anderson, White and Rettger, have replaced it by segregation methods, whilst in Germany a voluntary state-assisted scheme of control based on segregation of infected cows and their treatment with " entozon " was initiated in April, 1934.

Among the more recent efforts at vaccination or eradication reference maybe made to the following.

Seddon and Rose [1934] employed killed vaccines in a herd of about 100 cows over a period of five years. No controls were kept. The amount of obvious mastitis was reduced both in respect of numbers of cases and severity, but the incidence of streptococcus infection was unaltered. Similar findings were reported by Krage and Gipmann in 1931.

Plastridge, Anderson, White and Rettger [1934], working in five herds containing from 25 to 50 cows each, concluded that heat-killed vaccines did not adequately control streptococcus mastitis, while, on the other hand, hygienic measures and segregation of infected cows were found to be effective. From one herd the disease was said to have been eradicated and full details are promised later.

In 1933 we referred to attempts by other workers to control mastitis by segregation. Thus, Seelemann [1932] described the promising results obtained in two herds in which, purely by segregation of infected cows, infection was reduced respectively from 40 to about 10 per cent in fifteen months, and from 70 to 20 per cent in two years. In the latter herd it was reported [1933] that by combining entozon treatment with segregation the infection had been reduced to 4·2 per cent. Seelemann and Hadenfeldt [1933] reported on eleven other herds in which control of the disease had been attempted by segregation of cows found to be infected by three-monthly cultural examination of the milk, with or without subsequent treatment of infected cows with entozon. Their results are best given in the form of a table.

Herd	No. of Cows	Original Incidence (per cent)	Final Incidence (per cent)	Period	Control attempted by
S	177	42	29	2 years	Segregation alone
Ko	180	54	23	4 ,,	
D	65	60	47	2 ,,	
J	50	20	30	3 ,,	
L	138	43	30	3 ,,	
R	134	70	8·6	4 ,,	Segregation and 'entozon' treatment
Do	88	42	5·4	2 ,,	
H	81	52	10	2 ,,	
G	80	13	5	2 ,,	
F	22	100	0	5 ,,	
Q	200	45	7	2 ,,	

In all herds new infections continued to appear. It is seen that where entozon was used infection was considerably reduced and in one herd (F) was eradicated, whereas in herds in which segregation alone was adopted eradication was not achieved in any case and the incidence was only moderately reduced.

According to yet other reports from Germany, eradication or a striking reduction in the proportion of infected cows may be brought about by a combination of hygienic precautions in milking, with segregation and treatment of infected cows with entozon. The reports, however, do not show how often new infections arose, and, therefore, to what extent the reduction in the number of infected animals was due to continued treatment of old and newly infected cows or to the other measures used. The literature regarding the efficacy of entozon treatment will be dealt with in a subsequent report.

Methods used for the examination of milk samples

The methods used were similar to those given by Minett, Stableforth and Edwards [1933], to which reference should be made for details. They consisted essentially of: (1) The use of fore milk, after rejecting one or two streams.

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actually infected at December 31st, 1934, was 19 per cent, whilst at November, 1935, it was 13·1 per cent. In those infections which became permanent the degree of disturbance was slight at first, but during the last twelve months many of the cows concerned have shown symptoms characteristic of the chronic forms of the disease.

Herd A is also notable for the relatively large number of severe cases of mastitis due to other causes, and especially to streptococci belonging to Group II, which have occurred. In this herd, contrary to the almost general rule, such infections have been the cause of much greater loss than mastitis of the common chronic type.

HERD C

Herd C is made up of pedigree Jerseys and Red Polls, and from 1929, when observations commenced, it has carried over 100 head of milking stock. The herd has always been practically self-contained, though a few animals have been purchased from time to time. The herd is free from tuberculosis, certified milk being produced, and has been freed from contagious abortion by blood tests and segregation, coincidentally with the work reported here. Milking is carried out by hand.

HERD C

	State of Herd at end of Year			Additions, Removals and Cows which became Infected during previous 12 Months				
Year	Total	Healthy	Infected	Healthy became Infected	Healthy Added	Healthy Removed	Infected Added	Infected Removed
1929	86	53	34 (39·5)	—	55	26	3	18
1930	101	72	29 (28·7)	10 (29·4)	10	17	0	8
1931	86	61	25 (29·1)	4 (5·6)	43	7	4	15
1932	111	62	49 (44·1)	35 (57·4)	25	8	2	17
1933	113	68	45 (39·8)	11 (17·7)	31	12	0	16
1934	116	75	41 (35·3)	12 (17·6)	39	8	0	16
1935	134	99	35 (26·1)	7 (9·3)				13

For explanation see table for Herd A.

Of the 35 cows classed as infected in 1935, 11 had been free from infection for at least two years. The number of cows actually infected at the end of 1935 is, therefore, 24 and the real incidence 17·9 per cent.

Up to the end of 1931 mastitis-infected cows were kept in the same sheds as the uninfected, but segregated at one end and milked last. From 1932 onwards infected animals were removed to other sheds and milked by separate attendants. During the first four years heifers were milked after the older healthy animals until they had passed a test seven to ten days after calving. Since the middle

of 1932 they have been milked first of all.   In addition, the owner has always insisted that milkers should wash their hands after milking each cow and all reasonable precautions were taken to prevent spread from infected to healthy animals.

Complete herd tests of all animals in milk were carried out every three months in the case of the healthy group and at six- to twelve-monthly intervals in the infected group.   Samples were also examined seven to ten days after calving, and from any animal whose milk or udder became abnormal during the interval between herd tests.   The total number of animals in the healthy section of the herd in December, 1929, or which have since entered this section ("Healthy added") is 256.   Of these 256 cows, 72 were under observation for one lactation or less, 74 for two lactations, 47 for three, 32 for four, 24 for five and 7 for six lactations.   The number of tests made of each cow was from 2 to 17 and in one case 24, a total of 1,621 or an average for each animal of between 6 and 7.   The table shows the number of healthy and infected animals in the herd at the end of each year and the movements during the preceding twelve months.

The clinical severity of the disease originally present may be gathered from the following statements.   By the end of 1929 all the original milking herd had been tested, except four cows which were still dry.   Of the 34 infected cows present at that time, 17 were showing clinical symptoms or obviously altered milk in one or more quarters ; 8 showed abnormality of the milk only recognisable by laboratory examination, whilst in 9 the milk was unchanged.   The average number of infected quarters per animal was 2·4.

Comment

In this herd no special attempt was made to dispose of infected cows, but they were immediately segregated, and what were expected to be ample precautions were taken to prevent spread of infection.   Although there has always been a large group of infected cows on the farm in sheds adjoining those kept for healthy cows, it is somewhat surprising that new infections have continued to appear.   Thus, of the 256 animals which were passed as healthy when first tested in the period 1929-35, 79 had become infected up to the end of 1935.   Although dropping to less than 6 per cent in 1931 the annual percentage of new infections rose suddenly to over 50 in 1932.   It dropped again to 17 per cent in 1933 and 1934, whilst in 1935, 9 per cent have occurred.   The result is that the section described as infected, which was originally 39 per cent of the herd, still forms 26 per cent of it.   As in Herd A a number of animals apparently recovered.   These were, in nearly all cases, animals in which the infection was discovered only by means of the glucose broth enrichment medium and on only one occasion, in spite of numerous examinations during the succeeding years.   Since such cows have always been classed as infected the number of animals shown as infected is, in this herd also, unduly high for all years since 1932.   The real incidence at the end of 1935 was 17·9 per cent.

As in other herds, a certain amount of trouble due to mastitis streptococci of Groups II and III and to *C. pyogenes* has occurred, 18 cows in all being affected.

HERD I

At the end of 1930, when the first examinations were completed, Herd I consisted of about 160 head of pedigree milking stock of the British Friesian and Shorthorn breeds. It has been maintained since that time solely by means of home-bred animals. The herd is tuberculin-tested and it has been freed from contagious abortion coincidently with the work reported here. Milking is carried out by machine three times a day and all cows are stripped by hand.

After the first herd test infected animals were segregated as far as possible, and milked last. In 1931 the cows were arranged on three standings of which one contained only healthy cows, one healthy and infected at opposite ends, and one infected cows only, one man doing all the work on each line. Three milking machine units were in use in each row, separate units being reserved for the infected cows in the composite line. Teat cups were sterilised at the beginning of milking but not subsequently unless they were dropped. Towards the end of 1931 the owner decided to reduce his herd considerably and amongst those sold were most of the infected cows. From March, 1932, the few remaining infected cows were moved to other buildings.

HERD I

	State of Herd at end of Year			Additions, Removals and Cows which became Infected during the previous 12 Months				
Year	Total	Healthy	Infected	Healthy became Infected	Healthy Added	Healthy Removed	Infected Added	Infected Removed
1930	158	71	87 (55·1)	—	—	—	—	—
1931	125	76	49 (39·2)	16 (22·5)	60	39	7	61
1932	65	61	4 (6·2)	9 (11·8)	37	43	2	56
1933	61	61	0	0	31	31	0	4
1934	81	81	0	0	34	14	0	0
1935	79	79	0	0	18	20	0	0

For explanation see table for Herd A.

Milk samples were examined every three months. Samples were also taken from individual cows seven to ten days after calving, and from any animal which was giving altered milk or which showed any clinical abnormality of the udder or reacted to tests with brom-cresol-purple paper which were regularly carried out at the farm. The total number of animals passed into the healthy section of the herd in 1930 or which have since entered this section (" Healthy added ") is 251. Of these 251, 118 were under observation for one lactation or less ; 96 for two lactations ; 22 for three ; 10 for four, and 5 for five lactations. The number of tests made of each cow was from 2 to 22, totalling 1,476 or an average of about 6 for each cow.

The table, giving the changes in the herd year by year and its composition at December 31st of each year, shows that at the end of the third year the herd was free from *Str. agalactiæ* and has remained so since that time.

The mastitis originally present was of the characteristic chronic variety caused by *Str. agalactiæ*. The following data regarding the changes observed on one or more occasions will give some idea of its severity. Of the 96 cows of the original herd which were infected at their first examination in 1930 to 1932, 30 showed at or about the time of test clinical symptoms and obviously abnormal milk, 54 showed definite laboratory evidence of mastitis, whilst in 12 the milk was unaltered. The average number of infected quarters per cow was 2·6.

Comment

In Herd I the contagious form of streptococcus mastitis was radicated within a reasonable period in spite of the high original incidence (55 per cent) and the fact that for the first 18 months infected cows were housed in the same shed as the healthy. It is to be noted, however, that during the first 18 months many animals in the healthy group showed new infections, *viz.*, 22 per cent in 1931 and 12 per cent in 1932, a circumstance which there is reason to believe may have been due in some measure to slackness on the farm. There is no doubt, of course, that the eradication of the disease from this herd was facilitated very considerably by the decision of the owner in the autumn of 1931 to reduce his stock. Most of the infected cows were sold then or during the first half of 1932, and other infected cows were from that time completely separated.

Apart from mastitis due to *C. pyogenes*, which has caused the loss of a small number of quarters, the herd has been strikingly free from obvious udder disturbance due to streptococci other than *Str. agalactiæ* and during the last three years there has been practically no mastitis of any kind.

HERD J

This herd belonged to the same owner as Herds K and L, to be described next. Infected animals from Herds J and K were sent to other premises for isolation. There has been some interchange between the animals of Herds J and K, although different attendants were used on the two farms. Early in 1933 it was decided to raise a new herd on another farm, starting with freshly calved heifers. This is designated Herd L.

Herd J consists of about 45 animals of the Ayrshire breed and is maintained entirely by home-bred stock. It is free from tuberculosis; the eradication of contagious abortion was begun in 1932, and at recent tests there were no reactors. Milking is carried out by machine and all cows are stripped by hand.

The first tests for mastitis were carried out in November, 1931 and by the end of 1932, 55 animals of the original herd had been tested for the first time. Of these 55 animals, 16 (29·1 per cent) were infected.

All animals in milk were examined at intervals of three months during 1932 and subsequently at six-monthly intervals. All found to be infected at the first or later tests were removed as soon as convenient to the owner. Post-calving samples were not examined and animals found to be infected at any given test had, therefore, already been in the herd for some time. As mentioned, up to the end of 1932, 16 animals were recognised as infected at their first tests; in 1933 one such animal was located. The total number of animals classed as healthy at their first test in 1931 or subsequently was 92. Of these 92, 16 were under observation for one lactation or less, 32 for two, 20 for three and 24 for four lactations. The number of examinations made of each cow was from 2 to 11, a total of 464 or an average of about 5 for each animal. The table shows the number of animals in the herd at the end of each year and the movements during the previous twelve months.

The general character of the mastitis originally present, which was almost solely of the common chronic type, can be gathered from the fact that of the 16 cows of the original herd found to be infected, 4 showed at or about the time of test clinical symptoms or marked change in the milk, 9 showed definite laboratory evidence of mastitis, whilst in 3 the milk was unchanged in any way. The average number of infected quarters was 2.

HERD J

Year	State of Herd at end of each Year	Additions, Removals and Cows which became infected during previous 12 Months		
		Healthy became Infected	Healthy Added	Healthy Removed
1931	30	—	9	2
1932	31	6 (20·0)	34	7
1933	55	3 (9·7)	8	13
1934	45	5 (9·1)	11	8
1935	45	3 (7·0)		

For explanation see table for Herd A.

Comment

Although originally nearly one-third of the cows in this herd were infected, it is somewhat surprising that the infection has not been eradicated, since infected cows were removed to another farm. It is encouraging, however, that, whilst the number of animals in the herd has increased, the proportion of new infections dropped to less than 10 per cent in 1933 and 1934, and to 7·0 per cent in 1935. Failure to eradicate the disease may have been due to the relatively long intervals

between samplings and because newly added cows—many of which were infected—were not examined until the next herd test ; also because cows found to be infected were sometimes not removed for weeks or months. With one exception the herd has been free from mastitis due to causes other than *Str. agalactiæ*, whilst obvious mastitis due to this organism has disappeared, such infections as have occurred being detectable by laboratory methods only.

HERD K

This herd now consists of about 50 animals of the Friesian, Ayrshire and Guernsey breeds. It is maintained solely by homebred stock, and is free from tuberculosis. The eradication of contagious abortion was begun in 1932 and the herd is now free from reactors. Milking is carried out by hand.

Mastitis control was begun in November, 1931, and by the end of 1932, 93 animals of the original herd had been examined for the first time. Of these 93 animals, 50 (53·8 per cent) were infected.

HERD K

Year	State of Herd at end of each Year	Additions, Removals and Cows which became infected during previous 12 Months		
		Healthy became Infected	Healthy Added	Healthy Removed
1931	36	—	—	—
1932	27	16 (44·4)	7	0
1933	40	9 (33·3)	31	0
1934	55	4 (10·0)	30	11
1935	49	4 (7·8)	21	23

For explanation see table for Herd A.

All animals in milk were examined at intervals of three months during 1932 and subsequently at six-monthly intervals, those found to be infected at the first or subsequent tests being removed as soon as convenient to the owner. Post-calving samples were not examined and animals found to be infected at any given test had, therefore, been in the herd for some time. As already stated, 50 animals were found to be infected at their first test up to the end of 1932 ; in 1933 and 1934 the corresponding numbers were 7 and 3. The total number of animals classed as healthy at their first test in 1931 or subsequently was 125. Of these 125, 46 were under observation for one lactation or less, 39 for two lactations, 30 for three and 10 for four lactations. The number of examinations of each cow was from 2 to 10, a total of 480 or an average of nearly 4 for each animal. The table shows the number of animals in the herd at the end of each year and the events during the previous twelve months.

The general character of the mastitis originally present, which was almost solely of the common chronic type, can be gathered from the following data : of the 50 cows of the original herd found to be infected 8 showed, at or about the time of test, clinical symptoms or marked naked-eye changes in the milk, 31 showed definite laboratory evidence of mastitis, whilst in 11, the milk was unchanged in any way. The average number of infected quarters was 2·6.

Comment

Although since infected cows were removed, it might perhaps have been expected that the disease would by now have been eradicated, the fact that the number of infections has steadily fallen whilst the herd has been increasing is satisfactory. Thus, whereas the original percentage of infection was over 50 per cent, the percentages of new infections in 1932, 1933, 1934 and 1935 were 44, 33, 10 and 7·8 respectively. Failure to eradicate the disease may have been due to the same factors as operated in Herd J. The herd has been entirely free from mastitis due to causes other than *Str. agalactiae*, whilst mastitis due to this organism has been confined to latent cases.

HERD L

This herd was formed early in 1933 from freshly calved Ayrshire heifers belonging to the owner of Herds J and K. It is free from tuberculosis and contagious abortion. The animals are hand-milked.

Tests were carried out at intervals of six months and any infected animals at once removed. Post-calving samples were not taken. The total number of animals which have come under observation is 48, of which 13 have been in the herd for one lactation or less, 16 for two and 19 for three. During this period they have been examined 2 to 9 times—a total of 266 examinations or an average of between 5 and 6 for each cow.

The state of the herd in February of 1933 and at the end of each year and the events during the preceding twelve months are shown in the table.

HERD L

| Year | State of Herd at end of each Year | Additions, Removals and Cows which became infected during previous 12 Months | | |
		Healthy became Infected	Healthy Added	Healthy Removed
1933 (Feb.)	22	—	—	—
1933	32	1	12	1
1934	30	1	6	7
1935	31	0	8	7

For explanation see table for Herd A.

Comment

Of the 23 heifers drafted to this herd at the beginning of 1933, one was found infected at the first test. Twenty-six other heifers have been added and up to the time of this report two have been found to be infected. Although it was hoped that no infection at all would appear in this herd, the results are satisfactory so far as they go and show that when newly-calved heifers are kept apart from older cows the spread of *Str. agalactiæ* can be prevented.

Apart from one heifer which showed some disturbance in two quarters, due to Group III streptococci, the herd has been free from udder disturbance of any kind.

THE INCIDENCE OF INFECTION IN COWS OF DIFFERENT AGES

In Table 6 is given a summary of the data obtained at the beginning of our work in Herds C, I, J and K, to show the incidence of infection in cows of different ages. In these herds the original incidence of infection was 36·2, 55·1, 29·1 and 53·8 per cent respectively. Whilst the actual figures for age incidence vary somewhat in the individual herds, there is a gradual increase of infection with succeeding lactations, and it is seen that unless precautions are taken to protect heifers from infection as many as 30 per cent may become infected in their first lactation. These findings are similar to those of others, *e.g.*, Seelemann (1932).

TABLE 6

INCIDENCE OF *Str. agalactiæ* IN RELATION TO THE NUMBER OF LACTATIONS

Herd	1st Lactation			2nd Lactation		
	Total	Number affected	Per cent	Total	Number affected	Per cent
C	34	2	5·9	23	7	30·4
I	54	14	25·9	61	38	62·3
J	17	1	5·9	7	1	14·3
K	20	6	30·0	12	3	25·0
Total	125	23	18·4	103	49	47·6

Herd	3rd Lactation			4th to 10th Lactations		
	Total	Number affected	Per cent	Total	Number affected	Per cent
C	14	8	57·1	23	17	73·9
I	32	23	71·9	11	11	100·0
J	8	2	25·0	23	12	52·2
K	11	9	81·8	50	32	64·0
Total	65	42	64·6	107	72	67·3

DISCUSSION

In 1933, Minett, Stableforth and Edwards demonstrated the possibility of assembling a herd of cows free from *Str. agalactiæ* infection. The initial incidence of infection in the herd concerned (Herd B), which contained 49 cows, was 24·5 per cent, and in 1933 infection had been absent for about three-and-a-half years. At the present date the herd is still free.

In 1933, control work was also proceeding in the six herds now being discussed. It was hoped that by the use of similar measures it would be possible to eradicate the disease from these herds or, at least, to reduce the number of new infections to such a point that, with the normal disposal of animals as they got older, final elimination would be in sight.

In Herd I our hopes have been realised, since it has now been free from infection for nearly three years. Herds A, C, J and K on the other hand have continued to show new infections, although in all four herds the proportion has steadily fallen whilst the size of the herds has increased. With Herds J and K a number of circumstances have probably contributed to the failure to effect eradication. Thus, the intervals between herd tests had to be prolonged to about six months, post-calving samples were not examined, and freshly drafted cows were not examined at once, and, finally, it was impossible in some cases to remove infected animals to the separate farm for some weeks or months.

With Herds A and C there were several factors which increased the difficulties of control. Firstly, although the owners had prescribed strict precautionary measures for preventing spread of disease, a large infected section had to be maintained in the same or neighbouring sheds. Although it is difficult to see how the precautions taken could have been improved, the results, in conjunction with those of Herd I, suggest that the procedure of keeping infected and non-infected cows in the same or adjoining sheds is less satisfactory than the total removal of infected cows. This accords with the view of Seelemann [1933], that unless infected cows can be quickly evacuated, the removal of infection by suitable treatment is in most cases a valuable and perhaps essential aid to eradication. Secondly, in both herds the retention of *Str. agalactiæ* infected cows has helped to keep up the size of the infected group. Thus, in Herd A, there has been a large number of cases of clinically evident mastitis due to streptococci other than *Str. agalactiæ*, apparently arising in a sporadic manner. The *Str. agalactiæ* infections, on the other hand, were insignificant clinically, and it was not to be expected, therefore, that the owner would evacuate these in preference to the more obviously affected animals. In Herd C, it is to be noted that many infected cows were retained much longer than they would have been under ordinary circumstances to their high pedigree breeding value.

In Herds A and C the results at first appear to be disappointing. When the data are analysed, however, the results are less discouraging than they appear,

for the following reasons. It should be noted that in 1932 the laboratory control was made more strict and, also, that a selective enrichment medium was introduced capable of detecting very small numbers of streptococci in milk. The considerable increase in the number of new infections revealed in Herds A and C in 1932 may thus be accounted for. It follows at the same time that the incidence of infection prior to 1932 was probably higher than is represented by the figures given. Subsequent to 1932 the number of new infections fell, although not so much as was anticipated. In both herds a large number of new infections—mostly with non-hæmolytic *Str. agalactiæ*—were diagnosed solely by means of the enrichment medium and such animals were practically always giving normal milk. In a few cases chronic infections developed later or an obvious mastitis associated with large numbers of streptococci, but in nearly all cases infection was shown at one test and not at subsequent tests. In practice, however, such cows were always treated as infected and they appear as such in the tables, so long as they were still present in the herd. Thus, although the infected sections appear large in these two herds, many of the cows therein are not in fact infected at the present date. On the other hand, most of the cases which were diagnosed by direct plating of milk deposits have shown persisting infections. It should be said, finally, that the streptococci concerned in the transient infections mentioned were typical *Str. agalactiæ* biochemically and serologically and in some instances have been found capable of producing a chronic infection when injected into the udder of goats experimentally *via* the teat canal.

The experience in Herds A and C is of particular interest in establishing the fact that *Str. agalactiæ* is capable of setting up transient self-healing infections which can only be detected by the most stringent bacteriological tests. Since most of such infections were diagnosed by the enrichment medium only, there is some reason for regarding the use of an enrichment medium as superfluous for the control of the disease in practice.

Herd L remains for comment. This, it will be remembered, was formed from freshly-calved heifers and hence was expected to remain entirely free from *Str. agalactiæ*. As already stated, however, one heifer was found to be infected at her first test and two others subsequently became infected. Nevertheless, the disease is well under control and, although it is still too soon to conclude that infection will not appear later, the incidence of infection amongst these animals, most of which are now in their second or third lactation, need only be compared with that shown in Table 6 to realise that where chronic mastitis is at all prevalent the formation of a separate heifer herd is a measure of the greatest value. Emphasis has been laid in another article [Stableforth, 1935] on the practicability of forming a separate herd from young animals and the value of this procedure in protecting them from infection with other chronic contagious diseases besides mastitis.

SUMMARY AND CONCLUSIONS

An account is given of attempts in six herds to control chronic mastitis due to *Str. agalactiæ*. Latent infections were diagnosed by making cultural tests of the milk in blood agar plates or in a selective enrichment medium, these methods being supplemented by taking the chemical reaction of the milk and by noting the amount and character of the centrifuge sediment. These tests were made at three- to six-monthly intervals and in three herds also shortly after calving. Infected animals were milked after the healthy ones either in the same shed or in another shed on the same premises, or they were removed to another farm and milked by different attendants.

One herd (I) in which the original incidence of infection was 55 per cent has now been free for three years, a contributing result being the early sale of most of the infected cows. In three others (C, J and K) in which the original incidence was 40, 30 and 53 per cent respectively, the number of new infections has decreased during four to five years to 9·3, 7 and 7·8 per cent respectively in 1935. Herd L under the same ownership as Herds J and K was built up early in 1933 from freshly-calved heifers and is at present free. In Herd A, the incidence and numbers of new infections were at first low; the number of new infections rose in 1932, but since then has steadily fallen to 5·3 per cent in 1935.

The results are discussed in the light of the conditions prevailing in each herd and of the degree of control that could be exercised. For instance, in Herds J and K, although a separate isolation farm was used, the interval between tests could not be less than six months. In Herds A and C infected cows were kept in the same shed as the healthy or in sheds on the same premises, but in both herds, for reasons given, infected cows were retained far longer than would normally have been the case. Actually in Herds J, K, A and C the results are more encouraging than they appear. Thus, in general the new infections due to *Str. agalactiæ* have been insignificant clinically. Also, the real incidence of infection is at present less than the above statement implies because many cows, in which infection was diagnosed only by means of the enrichment medium and on one occasion only, have now been free from infection for at least two years although in practice they are still regarded as infected.

The experience in the present herds and in Herd B of the previous paper [1933] justify the following conclusions :—

(1) Eradication of contagious streptococcus mastitis may be achieved under certain conditions by the simple expedient of segregating infected cows, or, if that is not possible, by milking them last. The chances of success, however, are greatly increased if infected cows can be evacuated within a reasonable time. When this cannot be done, infusion of the infected udder with a suitable bactericidal agent should prove a useful aid.

(2) Whilst *Str. agalactiæ* in nearly all cases leads to permanent infection, it occasionally causes transient infections detectable only by the use of enrichment media.

(3) Experience has shown that the disease does not arise sporadically once all known sources of infection are removed. New infections must, therefore, be due to errors in milking or to deficiencies in methods of diagnosis.

(4) The formation where possible of a separate heifer herd is a measure of the greatest value.

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ABSTRACTS

Reproductive Hormone Therapy in Domestic Animals. G. H. HART AND H. H. COLE (1936). *J. Amer. Vet. Med. Assn.*, **88**, 12-23.

The authors have demonstrated the possibilites of harnessing the hormones which control the physiological process of reproduction in mammals for therapeutic application in correcting dysfunction of reproductive organs in both sexes in our domestic animals. The hormones concerned in reproduction are Gonadotropic hormone, Oestrin, Corporin, Male-sex hormone and Prolactin. Most of these hormones are ineffective when administered orally, as they are rendered biologically inactive in the gastro-intestinal tract.

The gonadotropic hormone is indicated in cases of impotency in males of any species of Mammalia as well as in females that fail to come in heat. This hormone is obtainable from the blood serum of pregnant mares from 37th to 200th day of pregnancy and from the urine of pregnant women. The authors used pregnant mare serum in their studies as in this material there is no danger of anaphylaxis in other species and no loss of potency in storage. This serum is readily standardized to a concentration of from 50 to 100 Rat units per cubic centimetre and it is very constant in the relative amounts of follicle-stimulating and luteinising principles. One rat unit is the amount that will produce from three to ten mature follicles in each of six immature female rats and half of which amount will fail to produce a vaginal smear of oestrum in a second group of six rats.

In ewes and sows 100 to 250 R. U. are sufficient and in the male, approximately double the dose for females is recommended. 750 R. U. are required to produce a physiological reaction in cows and mares. The authors claim good results by the use of this hormone on a stallion, boar and bull that were impotent. In wild animals in captivity, unresponsive males served their mates as early as 24 hours after injection.

Oestrin, now available on the market, in sufficient dosage stimulates the uterine musculature to most marked rhythmic contractions and for this reason can be used in causing the evacuation of the uterine contents in cases of pyometra and in increasing the epithelial cell activity in chronic metritis and vaginitis. The authors have not given any data regarding the indications and responses of *Corporin* and *male-sex hormone*.

Prolactin, also available in the market, is indicated in bitches or females of other species that fail to produce milk following parturition. However, this hormone has no place in increasing the normal milk flow of a lactating cow.

The authors remark that interesting and valuable results can be expected from the use of these products as therapeutic agents when the product to be used is carefully selected and judicially applied. [P. R. K.]

Die Stallrotkrankheit des Rindes. (Chronic Haematuria of cattle.)

Schlegal, M. (1934). *Munch. Tierartz Wchft.* 85, pp. 389-393, pp. 404, 408, and pp. 416-421 (with 7 illustrations).

In continuation of his first two articles of this series (Abstract in this *Journal* 6, 305), the author records observations upon aspects of heamaturia. In the light of the existing knowledge upon the mineral metabolism, role of vitamins, blood constituents and the physiological mechanism of clotting and retardation of clotting of blood of normal cattle, each of which has been discussed in detail, the author attempts to explain the causation of the disease and suggests tentative measures of control. The calcium and phosphorus content of blood serum of some healthy and 17 haematuria cases are estimated, and the figures from all the diseased animals are found to be low compared to the normals. The lowest, highest and the average values of calcium in haematuria are given as 6·95, 9·97, and 8·46 *mg.* per cent respectively and the corresponding figures of phosphoric acid as 11·8, 15·15, and 13·51 *mg.* per cent. On supplements of calcium and vitamins being given to the affected, these figures showed a rise, contrary to the case of the untreated controls—the calcium content of one of these controls dwindling down almost to zero. In spite of the favourable Ca : P ratio secured by supplements, some of the treated cases were found to be incurable, and the author ascribes this to the presence of advanced lesions in the kidney and the urinary bladder. Smears from the jugular blood were stained by different methods, and incomplete staining, the presence of broken erythrocytes, cells of unequal size, anisocytosis, and presence of diffuse basophile granules in red cells were noticed. Lymphycytes, basophile and acidophile cells were found to be normal but a considerable increase of the neutrophile leucocytes was observed. These examinations were carried out at weekly or fortnightly intervals during the whole course of the disease. While a gradual fall in erythrocyte count was recorded with the advance of the disease, normal values were regained by either effective treatment or removal from notorious localities. Cultural examination of the blood and urine for the presence of pathogenic bacteria and protozoa was carried out on various media under varying conditions but only negative results were obtained. In haematuria, the haemoglobin content of blood varied from 30 to 60 per cent of the normal. The blood was found to be more fluid with decreased coagulability, and this is ascribed to the calcium decrease, and absence of thrombogen and thrombokinase, which are destroyed by some toxic irritants from plants, which reduce the tone and elasticity of the blood vessels of the urinary system as a result of the paralysis of the vasoconstrictor nerves. The toxic substances mentioned are anemonin-camphor—the active principle of certain Ranunculaceae and of *Caltha palustris.*

Regarding the cure and control of the disease, the provision of fodder sufficiently rich in calcium and vitamins with the addition of large quantities of molasses has been recommended. Artificial manuring and dressing of soil, and drainage of land are suggested. Twenty-eight cows and five oxen, subjects of haematuria, were treated with different proprietary preparations including Vigantol, Chemosan, Clauden, Stryphnon, Vitakalk, etc. The action of Stryphnon was found to be comparable with that of Adrenalin in this disease, but more lasting results were obtained. Doses of 10, 20 and 40 c.c. of 1 per cent solution were given subcutaneously. E. Merck's

Ephedralin, in a 9 c.c. dose, administered once subcutaneously produced similar results. Of the above treated animals, 15 cows, and three oxen are stated to have been cured, while 10 cows and one ox had to be slaughtered.

The histopathological details, and the clinical symptoms described are in conformity with what is already known. [S. C. A. D.]

A new toxicant occurring naturally in certain samples of plant foodstuffs: IX. Toxic effects of orally ingested selenium. KURT W. FRANKE AND VAN R. POTTER (1935). *J. Nutrition* **10**, 213-221.

X. The effect of feeding toxic foodstuffs in varying amounts, and for different time periods. KURT W. FRANKE (1935), **10**, 223-230.

IX. Sodium selenite when fed to rats in doses corresponding to 22·3, 33·5 and 52·1 parts of selenium per million of a control wheat diet, developed symptoms of poisoning practically identical with those produced by the natural plant poison found associated with the protein fraction of some cereals. High levels of haemoglobin prior to a certain critical period (the ninth to seventeenth day of the experiment) followed usually by a distinct tendency towards anemia, voluntary restriction of food intake adversely affecting the rate of growth and profound pathological changes in the liver, spleen, kidneys and sometimes in the reproductive organs, are some of the main symptoms of selenium poisoning. The toxicity of selenium depends upon its ionic combination and although it has not been definitely proved by the authors that the toxicity of cereal grains is strictly proportional to their selenium content, the results support the view that selenium is closely connected with the natural toxicant.

X. Feeding experiments on rats with varying amounts of toxic grain have shown that even a concentration of 17·5 per cent of it in the diet produced depression of growth rates and caused deaths. As the percentage of toxic foodstuffs in the diet was increased, the consumption of food by the animals decreased, followed by retardation of growth rates, pathological lesions and deaths.

Rats kept on a diet containing toxic wheat for 30-, 20-, and 10-day periods resumed normal growth when they were changed to a control diet of safe grain, but the damage to the organs was never repaired. [A. C. R.]

CORRESPONDENCE

JOWAR (*SORGHUM VULGARE*) POISONING IN CATTLE

[The correspondence published below, regarding the article published in the December (1935) issue of the *Indian Journal of Veterinary Science and Animal Husbandry*, on "Jowar (*Sorghum vulgare*) Poisoning in Cattle" by G. K. Sharma, G.P.V.C., is of considerable practical importance. It is a well-authenticated fact that the sorghums and a variety of other fodder plants, including certain grasses, are poisonous to stock in certain circumstances. It is well, therefore, that stock-owners should be warned of the danger and that veterinarians should be informed of ready means of making an accurate diagnosis. From enquiries made by the Director of Veterinary Services, Punjab, at the Hissar Farm, it appears, however, that very large quantities of jowar have been fed to their cattle for many years without mishap. The matter is, therefore, one in regard to which an open mind should be kept but it is clear that the danger of poisoning by such plants should not be lost sight of.—EDITOR.]

To

THE EDITOR,

   Indian Journal of Veterinary Science and Animal Husbandry.

SIR,

  I notice with pleasure in the *Indian Journal of Veterinary Science and Animal Husbandry*, Vol. V. Part IV, December 1935, an article, "Jowar (*Sorghum vulgare*) Poisoning in Cattle" by G. K. Sharma, G.P.V.C. An article of this kind is very welcome to us of South India where the main fodder for cattle is *jowar* or *jola*, as it is called. Apart from what grazing (which is sometimes very poor) the cattle can pick up, they are fed on *jola, ragi* and paddy straws. During the growing season, however, green *jola* is fed in large quantities. So an article on this subject is of special interest to Mysoreans and will have effects all over Mysore State, if the advice is of real value. As I have been resident in Mysore State for more than ten years, I venture to offer some remarks on *jola* for feeding to stock.

  I have been in charge of this Palace Dairy Farm for the past seven years and can speak of personal experience in the matter. The average annual rainfall for this Farm and the surrounding districts is about 30 in., some years more and some years less. Mr. Sharma's article seems to have a special bearing on the conditions here in that every year drought-conditions prevail, this dry weather extending up to six and seven months, and the nature of the crop is conditioned by the good or bad rains that were precipitated, the crop being sometimes very stunted.

Now, according to Mr. Sharma's article, "When there is scarcity of rain, the formaldehyde by simple dehydration gives rise to prussic acid and water", which would lead to the inference that during droughty weather there is an abundance of prussic acid in the *jola* plant, which makes it unsafe to feed to stock. I have never heard, however, of serious casualties among cattle during this period.

The chief fodder for the cattle at the Farm is *jola* which we grow during the rains and is fed either green or as silage. There has never been a casualty so far through feeding this fodder. On the contrary, it makes excellent green feeding and silage, both of which maintain the milk-yield during times when there is little or no grazing in the pastures. This *jola* fodder is cut when the flowers open, *i. e.*, when its feeding value is at its highest. The cattle relish it very much and give good returns in milk.

A point of interest worth noting is that in Bangalore district the variety of *jola* grown is the red or "khaki" *jola*, while that grown in Mysore district and other parts of the State is the white variety, called "bili" *jola*, the botanical species being the same. I have grown and fed green to stock both varieties without any casualties, although the strength of the herd is over 320 head. There seems to be a difference of opinion as to one variety being more poisonous than the other. In any case even among the ryots, it is considered safe to feed green *jola* to cattle when the flowering stage has been reached, when the *jola* is said to have little or no prussic acid.

Another interesting point is that green *jola* can and has been fed to or grazed by cattle before the flowering stage has been reached without grave results. Noticing this feature I made enquiries of the local ryots as to why it is not dangerous to feed green *jola* before the flowering stage has been reached. The reply given me by an old stager was that *jola* is safe to feed when one or more internodes have formed on the stalk, which is in reality quite a young stage. Moreover, young ratoon *jola* (second growth that comes after the crop has been cut) has been grazed without dire results.

Experience in Mysore State shows that green *jola* can be fed safely to stock even if the plants have become stunted through drought, a condition that has been rather frequent within the past few years, in Mysore District alone. The ryots from long tradition, which comes of experience, know that green *jola* is quite safe to feed to stock. In fact, who has not commonly seen a ryot squatting on the ground holding his cattle by the nose-strings and pushing the green *jola* stalks from a bundle into the animals' mouths? No harm comes to these animals. This method of feeding is in fact to fatten and bring these animals into condition for show or sale purposes. The feeding of green *jola* thus shows that, contrary to experiences in the Punjab, there is absolutely no danger, but rather a gain in feeding this kind of fodder.

We, in Mysore State and South India in general, will become extremely alarmed if articles on sorghum poisoning should prohibit us from feeding stunted, green *jola* to cattle. If there have been no casualties through feeding green *jola*, whether well-grown or stunted—in fact it is more general to see short, stunted *jola* being grazed off rather than to permit it to ripen for harvest —it is not because sulphur or some other antidote has been administered as a preventive for cyanide-poisoning. The ryots' cattle will consider themselves extremely fortunate if they get a lick of salt ! Ryots as a rule are not given to spending money on modern or western veterinary medicaments, simply because they cannot afford it.

Why the Mysore cattle survive, even after eating green *jola*, seems to me a special field for investigation by biological chemists. The climate and soil of Mysore State will, in most probability, be one of a different nature from those prevailing in the Punjab, but the climatic conditions here seem to be excellent for cyanide poisoning according to the findings of Mr. Sharma.

In conclusion, I would like to say that until physiological and biological chemists and investigators can enlighten us more on the poisoning effects of hydrocyanic acid in green *jola* it seems to be safe for the Mysore ryots and animal husbandrymen to carry on, as they have done in past ages, of feeding green *jola* to their livestock, adopting the simple precautions of the past.

I shall be much obliged if you will kindly publish the above matter in your next issue in order that it may alleviate any fears that animal husbandrymen may be feeling regarding the feeding of green *jola* to cattle after reading the article on jowar poisoning.

Rayankere Dairy Farm,   Yours, etc.
The Palace, Mysore,   P. McIsaac, B.Sc. (Hons.),
Dated 27th January, 1936.   Ph. D. (Edin.)

To
    The Editor,
            Indian Journal of Veterinary Science and Animal Husbandry.
Sir,
    I am glad to receive the criticism on my paper " Jowar (*Sorghum vulgare*) poisoning in cattle" by Dr. P. McIsaac.

    Dr. McIsaac says that he has grown and fed green *jola* (*Sorghum vulgare*) without any danger to the cattle of Palace Dairy Farm, Mysore. He further adds that green *jola* can be fed to stock safely, even if the plants have become stunted through drought.

I quite agree with Dr. McIsaac that green healthy plants can be fed safely, and it is clearly mentioned in my article that normally it forms a wholesome fodder for animals. The point to discuss is whether the plant when wilted and stunted or under other abnormal conditions mentioned in the article, acquires toxic properties or not. Dr. McIsaac holds that it is absolutely safe to feed the cattle on sorghum under all conditions.

Now let us analyse the facts in the light of recent research and analytic works done on sorghum in India and abroad.

1. Tarantino, G. B. (1935) Toxicity of young sorghum, *Clin. Vet. Milano*. 58, 66-73.

Experiments were conducted to determine the variations in toxicity of the plant. One horse, two oxen and two goats were given a diet consisting solely of sorghum which had been grown for 30 days. On the 4th day of feeding the horse and oxen became ill and died unexpected in a short time.

2. Busso, L. (1934) Cyanogenetic plants, sorghum gentile poisoning in cattle. *Boll. 1st. Zooprofil, Sper, Turin*. March, pp. 34-46.

The cyanogenetic compounds occur in plants at the points of greatest metabolic activity and their formation is influenced by such factors as light, soil-conditions, moisture, etc. Varieties of sorghum have recently been grown in Italy for cattle-fodder and the author records several cases of poisoning. These plants contain a cyanogenetic glucoside and an enzyme which together produce a dangerous amount of prussic acid under certain conditions. In one case, two animals died and several others became ill after they had received a diet consisting solely of sorghum gentile. Young sorghum plants or those grown under unsuitable climatic conditions or on land which has been heavily manured with nitrates are likely to be dangerous.

3. In the Punjab it is commonly known as *sokar* (stunted) *jowar* poisoning. Col. G. K. Walker, the late Principal of the Punjab Veterinary College, Lahore, says in *The Veterinary Bulletin* No. 1921, "When crops of *jowar* suffer from want of water it is found that the leaves of the young plants elaborate a poison. If eaten by cattle the following symptoms are noticed. Dullness followed by tympanites, foaming at the mouth, etc. Death frequently results."

4. Coleman, F. F. (1934) carried out a comprehensive series of analysis for hydrocyanic acid (cyanogenetic glucoside) on several species of sorghum at various stages of growth. In all species hydrocyanic acid content decreased as the plants matured. (For details please see the *Rept. Dept. Agri.* 1933-34, pp. 144-147).

5. Peters, Slade, and Avery (1903). Poisoning of cattle by common sorghum and Kaffir corn. *Nebr. Agr. Sol. Bull.* 77 (*Experimental station U. S. A.*). They say that the poisonous character of the plant was due to the elaboration in it

of certain compounds capable of decomposing and yielding the poisonous hydrocyanic acid when masticated and taken into the stomach. A case of a heifer is described which dropped to the ground in ten minutes after having been driven into a sorghum field.

6. Wittman, J. J. and West, R. M. (1915). Notes on the hydrocyanic acid contents of sorghum. *Jour. Agric. Research*, Vol. 4, p. 179. They have shown that inadequate water supply is usually accompanied by high prussic acid content.

Avery says that the amount of hydrocyanic acid is greater in stunted plants. Maxwell believes that soil rich in nitrogen produces plants rich in the glucocides.

I would quote a few more experimental works done in the neighbouring province of Dr. McIsaac, *viz.*, Madras.

7. Acharya, C. N. (1933) *Indian Jour. of Agricultural Science*, Vol. III, Part V, pp. 851-868. Investigation on the development of prussic acid in *cholam* (*Sorghum vulgare*). He commences his article with "That *cholam* (*Sorghum vulgare*) under certain conditions of growth becomes highly poisonous for cattle, has been known for a long time past." He analysed the plant by three different methods and found the highest percentage of prussic acid in stunted *Sorghum vulgare* plants (*vide* Table 3, page 855). He further mentions that a field of Periamanjal *cholam* (sorghum) suffering from serious drought and highly stunted (Field No. 77) was taken up for examination, and its prussic acid contents compared with that of *cholam* growing in the adjacent part of the same field, which had been supplied with drain water and had grown luxuriantly. The stunted plants showed nearly 6 to 7 times the percentage of prussic acid. This effect of drought was examined in a number of other fields also, and in each case it was noticed that drought is one of the factors promoting the accumulation of prussic acid in *cholam*.

8. Bensen and Sobha Rao (1906) *Bull. Dept. of Agriculture, Madras*, No. 55. *Cholam* (*sorghum vulgare*) is largely used as cattle fodder throughout India, and several deaths of stock have been reported from time to time.

Space does not permit of more quotations from the works of different authors, but as Dr. McIsaac is interested in the subject, I give the following references for his information.

1. Slade, H. B. (1903). Prussic acid in sorghum. *Jour. Amer. Chem. Society*, Vol. 25, No. 1, p. 25.

2. Pinckey, R. M. (1924). Effect of Nitrate application upon Hydrocyanic acid content of sorghum. *Jour. Agric. Research*, Vol. 27, p. 717.

3. Pease, H. T. (1897). Poisoning of cattle by *Andropogon sorghum. Jour. of Comp. Med. and Vety. Arch.*, Vol. 18, p. 679

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4. Maxwell, W. (1903). Sorghum poisoning. *Queensland Agric. Jour.* Vol. 13, No. 5, p. 473.

5. Alway, F. I. and Trumball, R. S. (1909). Occurrence of Prussic Acid in sorghum and maize. *Nebr. Agr. Expt. St. 23rd. Ann. Rep.*, p. 35.

6. Belfour, A. (1903-4). Cyanogenesis in *Sorghum vulgare*. *First Report Welcome Res. Lab. Gordon Mem. Coll. Khartoum*, p. 46.

7. Francis, C. K. (1915). Poisoning of Livestock while feeding on plants of the sorghum group. *Okla. Agr. Exp. Stn.* Sl. Cix Imp. 38.

8. Avery, S. (1902). Laboratory notes on poison in sorghum *Jour. of Compar. Med. and Vet. Arch.* Vol. 23, No. 11, p. 704.

9. Dr. Steyn observed in a case of *Cynodon transvaalense* where the plant growing in moist conditions yielded no prussic acid, whereas the plants growing on a dry ridge showed a strong reaction.

10. Dr. A. C. Leechmann in the *Onderstepport Jour. of Vet. Sc. and Anim. Ind.* July, 1935, gives a long list of 88 grasses containing prussic acid or cyanogenetic glucoside. In the list six varieties of sorghum have been mentioned, and *Sorghum vulgare* is one of them. In this article entitled " Hydrocyanic acid in grasses " the author has discussed in detail the influence of climatic conditions, wilting, stunting, drought and soil, etc., on the production of hydrocyanic acid and the toxicity of the plants.

As *Sorghum vulgare* has been shown to be toxic, under certain conditions, in several parts of the world, it would appear reasonable to expect similar conditions in Mysore.

I would, however, like to make a few suggestions regarding this difference found in Mysore.

The following points deserve serious consideration :—

1. The most prominent symptom is tympanites and when death occurs in sorghum poisoning it is usually attributed to this or to some acute disease such as H. S.

2. Duration of Hydrocyanic acid poisoning is short. The cases are seldom brought to the hospital and thus a veterinarian often does not get an opportunity to diagnose and treat a case.

3. If any cases are seen the diagnosis will probably be " fodder poisoning." This is a vague term and steps to have the fodder and ingesta analysed are rarely taken.

4. The analysis of the stunted plants should be carried out for the identification of hydrocyanic acid and in cases reported to have died of tympanites the ingesta should be examined.

The beginning of my paper is "The subject of plant poisoning in animals has received very little attention in India, which is mainly due to the difficulty in its diagnosis from the clinical symptoms alone, and the fact that majority of field veterinarians are unable to secure the collaboration of a chemist for analysis of suspected sample of fodder or ingesta." In my article, stress is laid on chemical tests and a simple test for the detection of hydrocyanic acid is given which is suitable for adoption under field conditions. This would be of a great help in the diagnosis of hydrocyanic acid poisoning. This is not a fact that cases of sorghum poisoning do not occur but they remain undiagnosed. It is regretted that Dr. McIsaac has taken my paper as extremely alarming. I wish to emphasize that it was written merely as a warning against the feeding of wilted and stunted sorghum plants. This warning is not from me only but also from leading research workers and chemists working in the Agricultural and Veterinary Departments in India and abroad. It is especially significant when Mr. Acharya, working in an adjoining province found the highest percentage of hydrocyanic acid present in the stunted sorghum of Madras. What is true of Madras and other countries should apply to Mysore, provided the same conditions favouring the production of hydrocyanic acid exist.

Further Dr. McIsaac says that ryots are unable to spend money on sulphur. The stunted *jola* should be dried in the shade to eliminate hydrocyanic acid before feeding it to cattle. They can certainly do this much, which costs nothing. (Please see my article, page 377*).

In South Africa a disease (Geilsiekte) affecting sheep and cattle had been known for a long time, and Brown reported this in 1864, and MacOwen referred to heavy losses caused by this disease in 1877. This condition remained undiagnosed for a long time even in the hands of experts. It was Dr. Steyn who discovered the actual cause of Geilsiekte, for the first time in 1929, to be prussic acid poisoning due to the ingestion of wilted grasses. It is, therefore, not at all surprising if cases of sorghum poisoning remain undiagnosed in Mysore or elsewhere.

It is not essential that stunted and wilted sorghum fed to cattle may prove toxic or fatal in all cases. There are certain factors which influence the toxicity of sorghum. (For details please see my article, page 377*). It is important both from professional and livestock viewpoints to test the stunted and wilted Jowar, especially when it is young, for the presence of hydrocyanic acid. Cattle having been fed on such sorghum and showing symptoms of poisoning or having died of tympanites within a few hours, should be suspected of hydrocyanic acid poisoning. The ingesta should be examined by the picrate method mentioned in my article (page 379*) or sent to the Chemical Examiner.

Yours, etc.,

G. K. SHARMA.

* Published in the *Indian Journal of Veterinary Science and Animal Husbandry*, Vol. **V**, Pt. IV, December, 1935.

NOTE

CATALOGUE OF BRITISH MEDICAL FILMS.

We have received a copy of the " Catalogue of British Medical Films of Technical Interest to Medical Practitioners and Students " (Price 1s.) prepared by the Medical Panel of the British Film Institute, 4, Great Russel Street, London, W. C. 1. It has been classified, so far as existing films permit, in accordance with the medical curriculum. Full indices have been included for ease of reference and particulars are given of name of author, width of film, silent or sound, length, suitability, name and address of owner. It is hoped that the catalogue will be found useful to those who wish to make use of films of medical interest. [EDITOR.]

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The Indian Journal of Veterinary Science and Animal Husbandry

Editorial Committee

The Editorial Committee, in its work of examining papers submitted for publication, is assisted in an honorary capacity by a large number of scientists working in various parts of India.